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Randomized Study for the Treatment of Adult Advanced Hodgkin's Disease: Mechlorethamine, Vincristine, Procarbazine, and Prednisone (MOPP) Versus Lomustine, Vinblastine, and Prednisone[1]

Howard A. Liebman,* Gilbert J. Hum, William W. Sheehan, Victor M. J. Ryden, and Joseph R. Bateman[2,3]

Forty-seven patients with advanced Hodgkin's disease were entered in a prospective, randomized trial comparing MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) with a regimen containing lomustine (CCNU), vinblastine, and prednisone (CCNU-VP). Both groups were comparable for the variables of age, stage, substage (symptoms), histology, prior radiation, and sites of involvement. Seventy-two percent of CCNU-VP-treated patients achieved a pathologically documented complete remission (CR) compared to 41% of the MOPP-treated group. Two additional patients treated with MOPP had remission documented only clinically but have been long-term, disease-free survivors. There was a greater frequency of CR in the patients who had received previous irradiation when compared to patients with no prior irradiation. After a median follow-up of > 89 months, there is no statistical difference between the two treatment groups in survival (45% for MOPP and 60% for CCNU-VP). Further, no statistical difference in survival for the two treatment groups was noted when compared by histology, stage, or symptoms. The CCNU-VP combination was better tolerated with significantly less nausea and emesis. The alternative drug regimen of CCNU-VP appears to be as effective as MOPP in producing CR and long-term survival in patients with advanced Hodgkin's disease. [Cancer Treat Rep 67:413–419, 1983]

Combination chemotherapy has become the treatment of choice for patients with advanced Hodgkin's disease or for patients who relapse after radiotherapy (1,2). The combination of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) is most commonly used and produces complete remissions (CR) in approximately 70% of the patients treated. Of the patients who achieve a CR, > 60% are alive with no evidence of disease at 10 years (2). Alternative drug combinations have been studied with previously untreated patients and are reported to produce an equivalent number of CR (3–6). The existence of these alternative combinations is important because a significant number of patients either fail to respond to MOPP or tolerate MOPP poorly. Unfortunately, long-term follow-up and survival data have not been reported for these combinations and it remains to be determined whether they are equivalent to MOPP in their curative potential.

In 1973, the Western Cancer Study Group (WCSG) initiated a randomized trial of MOPP versus lomustine (CCNU), vinblastine, and prednisone (CCNU-VP) for the treatment of advanced Hodgkin's disease and for patients who had relapsed following radiation therapy. This combination of agents was selected because of the significant single-agent activity against Hodgkin's dis-

[1]Received Feb 1, 1982; revised Mar 4, 1983; accepted Mar 22, 1983.

[2]This study was conducted by the Western Cancer Study Group. The following members participated in the study: H. Liebman, G. Hum, V. Ryden, and J. Bateman, Los Angeles County-University of Southern California Medical Center; J. Block, Harbor-UCLA Medical Center, Torrance, CA; J. Brook, Long Beach Veterans Hospital, CA; D. Grant, USAF Medical Center, Travis AFB, CA; R. Bohannon, San Francisco General Hospital, CA; C. Hendrickson, Kaiser Permanente Hospital, San Francisco, CA; J. Linman, University of Oregon, Portland; R. Hestorff, Kaiser Permanente Hospital, Oakland, CA; A. Glass, Kaiser Permanente Hospital, Portland, OR; R. Mass, Portland Veterans Hospital, OR; J. Lewis, University of California, Davis; C. Kardinal, Ellis Fischel Cancer Hospital, Columbia, MO; and W. W. Sheehan, University of Texas, Dallas.

[3]We thank Alexandra M. Levine, MD, for her review of this manuscript, and also Rebecca A. Skibinski for her help in preparing the manuscript.

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ease reported for each of the drugs, and because their use in combination could potentially result in reduced toxicity when compared to MOPP. A preliminary report of the study demonstrated equivalent numbers of CR for both combinations, but better patient tolerance and reduced toxicity for CCNU-VP (7). We now report the long-term follow-up for these patients.

METHODS

Patient Population

Fifty-five patients were entered in the study from August 23, 1973, to February 28, 1978. Eight patients were excluded from the final evaluation; three had non-Hodgkin's lymphomas on pathologic review, four had significant protocol violations, and one had incomplete data to document response. Three of the five patients with Hodgkin's disease excluded from final evaluation are alive with no evidence of disease at the time of this report. The remaining 47 patients are reported herein. All data were analyzed as of January 1, 1982. All biopsies were reviewed by one of the authors (W. S. Sheehan), and were classified according to the Rye conference modifications of the Lukes and Butler classification. Patient characteristics are shown in table 1.

TABLE 1.—Pretreatment patient characteristics*

Characteristic	MOPP	CCNU-VP
Patients eligible	22	25
Mean age in yrs	30.7	37.3
Sex (male/female)	18/4	15/10
Histology		
Nodular sclerosis	10	14
Mixed cellular	8	8
Lymphocyte depletion	4	3
Stage		
II	3	2
III	7	12
IV	12	11
Substage		
A	6	5
B	16	20
Prior irradiation	11	11
Site of involvement		
Liver	5	9
Spleen	9	12
Bone marrow	6	2
Lung	7	3
Skin	1	2
Bone	1	1
Lymph node	18	24
Mean hemoglobin (g/dl)	10.7	11.8
Mean Karnofsky performance score	75.5	78.2

*Unless otherwise specified, values = No. of patients.

Staging

The Ann Arbor staging classification was used and the reported stage reflects extent of disease at time of entry in the study. Complete clinical and pathologic staging included medical history, physical examination, and hematologic and laboratory studies. The laboratory studies obtained included: cbc with differential, alkaline phosphatase, SGOT, SGPT, total and direct bilirubin, total protein and albumin, BUN, creatinine, serum electrolytes, and urinalysis. Radiologic studies included chest radiograph, pulmonary tomography if indicated, skeletal survey, and bipedal lymphangiogram. Gastrointestinal studies were performed if clinically indicated. Radionuclide studies included liver-spleen scans and whole-body gallium scans in most patients. Posterior iliac crest bone marrow aspiration and biopsy were performed in all patients. Laparotomies were performed in 22 patients to confirm stage. The pretreatment pathologic stages of the patients in each treatment group are shown in table 1.

Treatment

Informed consent was obtained from all patients prior to treatment. Patients were randomized to either treatment arm by the WCSG Studies Analysis Center. The drug dosages, treatment schedules, and dose modifications for both arms are shown in table 2. The dose of CCNU was escalated by 10 mg/m^2 with each successive course up to a maximum dose of 120 mg/m^2, provided the patient's wbc count was $\geq$ 4000/mm^3 and platelet count was $\geq$ 100,000/mm^3 at the time of the next course. Both treatment arms were to be given for a minimum of eight courses for a CR or until evidence of disease progression. Patients who failed to respond, or who relapsed on treatment arm, were to be crossed over and

TABLE 2.—Chemotherapy dose and schedule

Drug	Dose* (mg/m^2)	Schedule (Days)
MOPP (4-wk cycles)		
Mechlorethamine hydrochloride	6	1 and 8
Vincristine	1.4	1 and 8
Procarbazine	100	2–15
Prednisone	40	2–15
CCNU-VP (6-wk cycles)		
CCNU	90	1
Vinblastine	6	1 and 8
Prednisone	40	2–15
No therapy		16–42

*Dose modifications (cells/mm^3): wbcs $\geq$ 2000 and/or platelets $\geq$ 100,000, give 100% of all drugs; wbcs = 1500–1999 and/or platelets = 75,000–99,999, give 50% of all drugs except vincristine; wbcs = 1000–1499 and/or platelets = 50,000–74,999, give 25% of all drugs; and wbcs < 1000 and/or platelets < 50,000, give *no* drugs. Vincristine doses are discontinued for grade 3 or 4 neurotoxicity.

TABLE 3.—% of total calculated drug dose*

Treatment arm	% of calculated dose, by response		
	None	PR	CR
CCNU-VP			
Prednisone	100	96	98
Vinblastine	96	97	94
CCNU	97	87	93
MOPP			
Prednisone	92	82	100
Vinblastine	94	82	84
Mechlorethamine	100	85	82
Procarbazine	92	78	74

*Average of individual patient drug doses through 8 courses of therapy.

treated with the alternative regimen. Unfortunately, too few patients were so treated to allow evaluation of cross-over therapy.

Restaging and Response Criteria

Standard WCSG response criteria were employed. A CR was defined as the complete disappearance of all evidence of Hodgkin's disease, including correction of all abnormal pretreatment studies, and no evidence of Hodgkin's disease with repeat biopsies of previously involved sites. Patients without biopsy confirmation of their CR were called partial responders. A partial remission (PR) was defined as a $> 50\%$ reduction in the size of all measurable lesions. Patients with $< 50\%$ reduction of lesions, stable disease, or progressive disease are considered to be nonresponders. Toxicity was assessed using standard WCSG toxicity criteria.

Statistical Analysis

Curves of the probability of survival were calculated from the date of beginning therapy by the method of Kaplan and Meier (8). Statistical significance for survival curves was determined by the log-rank test.

RESULTS

There were no significant differences between the two treatment groups for the variables of age, stage, substage (symptoms), histology, prior irradiation, or sites of involvement (table 1). Twenty-two patients received MOPP, while 25 patients were treated with CCNU-VP. All 47 patients completed at least one course of therapy, but only 13 patients treated with MOPP and 17 patients treated with CCNU-VP received at least six full doses of therapy. Three patients treated with MOPP and four patients treated with CCNU-VP died before six courses of therapy. For the ten remaining patients who received fewer than six courses of therapy, there was no difference in survival when compared to the 30 patients who received more than six courses. There was no statistical difference between groups in the percentage of total calculated drug dose given through eight courses of therapy (table 3).

The response frequencies for each treatment group are shown in table 4. With CCNU-VP therapy, 72% (18 of 25 patients) achieved a pathologically documented CR, compared to 41% (nine of 22) for MOPP therapy ($P = 0.037$). Two patients treated with MOPP obtained clinical CR but are listed as partial responders because the necessary biopsy specimens were not obtained. Both patients remain in remission at the time of this report, and because of prolonged disease-free survival, they are included in the curve for disease-free survival. There was no statistical difference in the frequency of CR when compared by stage or symptoms. Six of 11 patients (four treated with CCNU-VP, two treated with MOPP) without symptoms achieved a CR compared with 21 of 36 patients (13 treated with CCNU-VP, eight treated with MOPP) with symptoms. However, there was a greater frequency of CR in patients receiving

TABLE 4.—% of response to MOPP and CCNU-VP

Treatment arm	Response		
	None	PR	CR
MOPP	3 (13)	10 (45)*	9 (41)
CCNU-VP	4 (16)	3 (12)	18 (72)

*Includes 2 patients with clinical CR, but no pathologic confirmation.

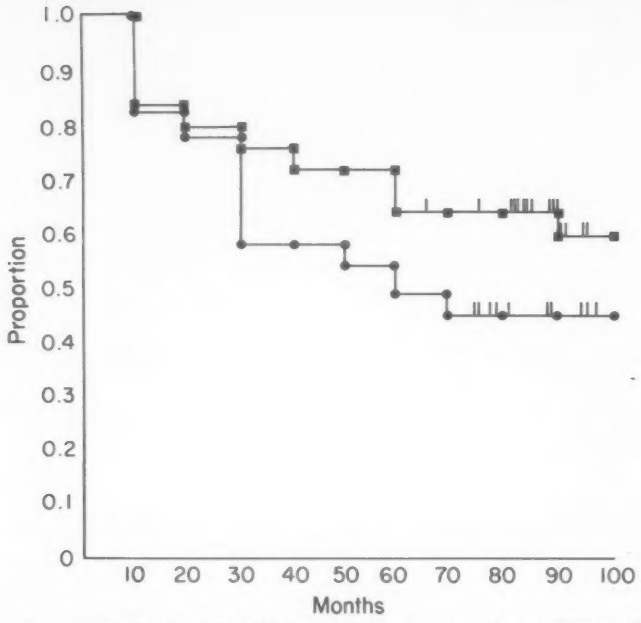

FIGURE 1.—Comparison of survival between the 25 patients treated with CCNU-VP (■) and the 22 patients treated with MOPP (●); $P = 0.41$.

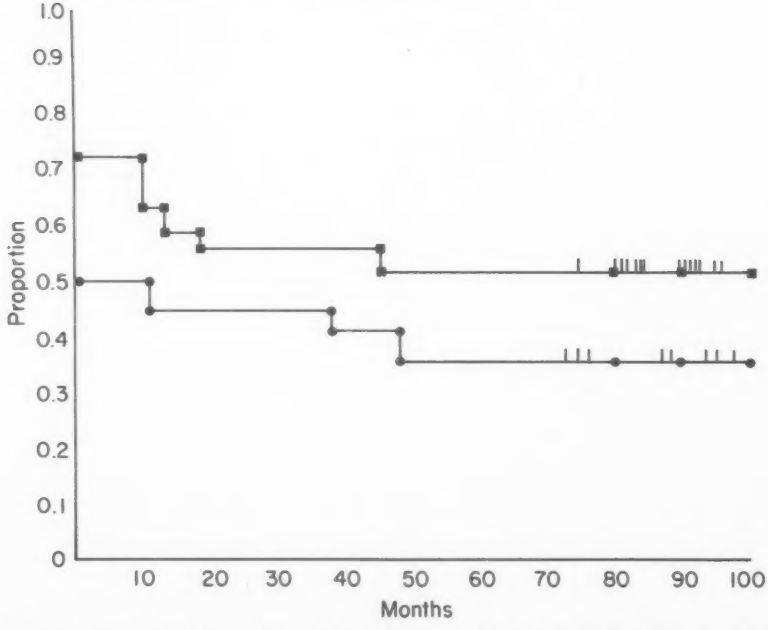

FIGURE 2.—Comparison of disease-free survival for all patients, including the 25 patients treated with CCNU-VP (■) and the 22 patients treated with MOPP (●). ($P = 0.52$ for the comparisons between the MOPP- and CCNU-VP-treated patients.) Included are 2 patients treated with MOPP reported as partial responders but who remain in remission with no evidence of disease (*see* Results section).

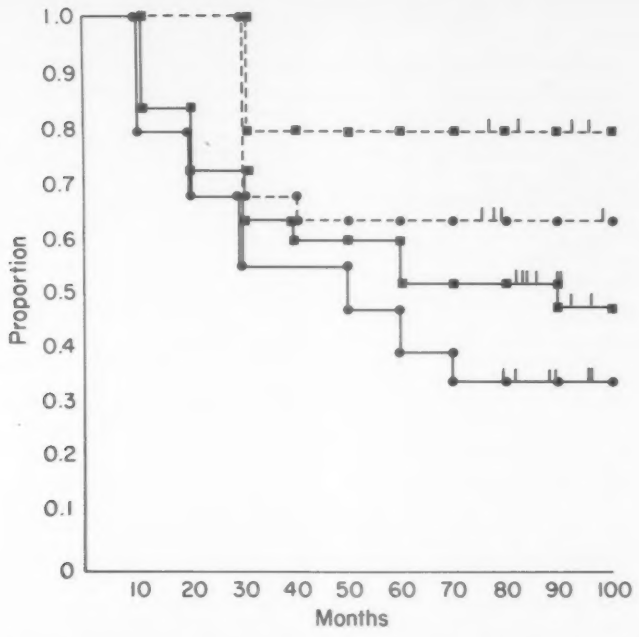

FIGURE 3.—Comparison of survival between the asymptomatic patients (broken line) and the symptomatic patients (solid line). There were 5 asymptomatic patients and 20 patients with B symptoms treated with CCNU-VP (■). There were 6 asymptomatic patients and 16 patients with B symptoms treated with MOPP (●). (Comparison of both regimens for the asymptomatic patients, $P = 0.72$, and for the symptomatic patients, $P = 0.62$.)

previous irradiation (17 of 22 patients) when compared to patients with no prior radiation (ten of 25, $P = 0.034$). There was no correlation between the percentage of total drug dose received and the resulting response for either treatment group (table 3).

Survival for both treatment groups was calculated from start of treatment to January 1, 1982, and is shown in figure 1. The median survival time for the patients treated with MOPP was 52 months, while the median survival time for the patients treated with CCNU-VP has not yet been reached. There was no statistical difference in survival between either treatment group ($P = 0.41$). In addition, there was no difference in survival between either group when compared by pathologic stage (III or IV). Figure 2 shows relapse-free survival for both treatment groups. The relapse-free survival for the patients treated with CCNU-VP is 52% and for those treated with MOPP is 36%. There was no statistical difference in survival for any histologic group. Survival for patients from both treatment groups without symptoms was better than survival for patients with B symptoms, although this was not statistically significant (fig 3). At the time of this report, there are 26 patients alive, of whom 15 (60%) were treated with CCNU-VP and ten (45%) with MOPP. Three patients are alive with disease. One patient with stage IIIB mixed cellular disease, who was treated with CCNU-VP, relapsed after a CR of 46 months. Two other patients treated with MOPP have relapsed after CR with stage IV lymphocyte depletion disease.

Toxicity

The treatment groups were compared through the first six courses of therapy for the incidence and severity of leukopenia, thrombocytopenia, gastrointestinal toxicity, and nervous system toxicity (table 5). There was no statistical difference between the two groups in the incidence or severity of leukopenia or thrombocytopenia. Cumulative marrow toxicity was not observed in the group treated with CCNU-VP. There was a greater incidence of peripheral neuropathy in the group treated with MOPP, although not statistically different. Significantly greater nausea and emesis was reported in the patients treated with MOPP. Two patients treated with MOPP refused further therapy after one and two courses because of this complication.

TABLE 5.—Total episodes of toxicity reported*

	No. (%) of episodes					
	CCNU-VP			MOPP		
	None	Grades 1–2	Grades 3–4	None	Grades 1–2	Grades 3–4
Leukopenia	69 (65)	29 (27)	8 (8)	40 (45)	40 (45)	9 (10)
Thrombocytopenia	86 (87)	10 (10)	3 (3)	70 (89)	9 (11)	0 (0)
Gastrointestinal effects†	72 (75)	24 (25)	0 (0)	48 (61)	22 (28)	9 (11)
Neurologic effects	77 (79)	21 (21)	0 (0)	63 (69)	24 (27)	4 (4)

*Total for 6 courses of therapy. Presence or absence of toxicity not reported for some individual patient courses. Standardized WCSG toxicity criteria were used.

†CCNU-VP vs MOPP ($P < 0.01$).

Mortality

At the time of this report, 22 patients are dead. Eighteen patients died with active disease; 13 of these patients never attained a CR. Two patients, one from each treatment group, died early, secondary to drug-induced leukopenia with complicating sepsis. Four patients died with no evidence of Hodgkin's disease, as confirmed by autopsy. One of these patients died of congestive heart failure 50 months after cessation of therapy. Another patient committed suicide 53 months after therapy. Two of these patients died of second malignancies: acute nonlymphocytic leukemia and large cell lymphoma. Both patients had been treated with MOPP and had received prior irradiation.

DISCUSSION

MOPP has become the standard treatment for advanced Hodgkin's disease (2). Despite its effectiveness, a significant number of patients treated with MOPP will die of active disease. Therefore, there is a need for effective alternative therapeutic combinations for patients who fail MOPP or poorly tolerate its significant toxicity.

In this prospective randomized trial, CCNU-VP was shown to be superior to MOPP in producing CR and equivalent to MOPP in achieving long-term survival. The CR rate of 41% in this study for the patients treated with MOPP is significantly lower than the CR rate reported in other trials. The requirement in this study for pathologic-biopsy documentation of CR may account for this discrepancy. Two patients treated with MOPP were called partial responders because the necessary biopsy specimens were not obtained to confirm their clinical CR. However, the CR rate of 72% obtained in the patients treated with CCNU-VP is equivalent to that reported for MOPP in other series (2,9), and 60% of the patients treated with CCNU-VP are alive after a median follow-up of > 89 months.

Drug combinations employing CCNU have previously demonstrated significant activity in both untreated and previously treated patients. A randomized Cancer and Acute Leukemia Group study of CCNU, vinblastine, procarbazine, and prednisone compared to MOPP with untreated patients reported a superior response rate of 66% for the CCNU-containing combination (10). Diggs et al (11) reported 15 CR among 20 untreated patients given a combination of CCNU, doxorubicin, bleomycin, and streptozocin. However, long-term follow-up and survival for the patients treated with these combinations have not been reported.

The CCNU-VP combination was well-tolerated, with significantly less nausea and emesis than MOPP. There was no statistical difference between CCNU-VP and MOPP in the severity of leukopenia or thrombocytopenia. Another WCSG study of non-Hodgkin's lymphomas comparing vincristine, prednisone, and cyclophosphamide with vincristine, prednisone, and CCNU (CCNU-OP), both given as 6-week cycles, reported significantly greater thrombocytopenia with the CCNU-containing combination (12). The reason for this discrepancy in incidence of thrombocytopenia may be due to the lower CCNU starting dose of 90 mg/m^2 in the CCNU-VP combination compared to the starting dose of 130 mg/m^2 in the CCNU-OP study. In the CCNU-VP study, 14 of 27 patients had dose escalations of CCNU, but no patient received a dose > 110 mg/m^2.

The most serious late complication of MOPP therapy appears to be the development of second malignancies (13–15), particularly acute leukemia or large cell lymphoma. Two patients in this study, treated with MOPP after radiation therapy, have died of second malignancies. In contrast, no patient in the CCNU-VP-treated group has developed another malignancy. Although the small number of patients in this study prevents statistical analysis of this problem, an important future consideration in evaluating alternative combinations should be possible late carcinogenic or leukemogenic effects.

Three patients treated with CCNU-VP developed bi-

lateral aseptic necrosis of the femoral heads. These three patients received their therapy at different institutions. None had received pelvic radiation or were taking other medications associated with this complication. The association between steroid use and aseptic necrosis is well-documented (16), but this complication is rarely reported with the intermittent use of prednisone as given in MOPP or CCNU-VP. In addition, the cumulative dose of prednisone in the CCNU-VP combination was less than that received with the MOPP combination. Without evidence of other contributing risk factors, it appears that the CCNU-combination may be associated with an increased risk of aseptic necrosis.

In this study, the only statistically significant prognostic factor was the positive effect of prior radiotherapy. A similar observation was made in the National Cancer Institute (NCI)-MOPP study (2), with a CR rate of 94% for the patients who received prior radiotherapy compared to a CR rate of 78% for the nonirradiated patients. Why previously irradiated patients had a better response to both MOPP and CCNU-VP is unknown, but it may be due to a smaller tumor burden at the time chemotherapy was begun. Several other prognostic factors associated with decreased survival were reported in the NCI-MOPP study, including B symptoms, nodular sclerosis histology, and stage IV disease. The small number of patients in this study prevented statistical analysis of these prognostic factors.

The long-term follow-up of the MOPP-treated patients at the NCI confirms earlier projections of durable, prolonged, relapse-free survival for patients who achieve CR. The present study confirms the NCI observations and demonstrates that alternative drug combinations capable of producing CR in a high percentage of patients are also capable of producing durable remissions and prolonged survival times. This report should also lend support for continued investigations of alternative combinations for the treatment of advanced Hodgkin's disease.

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Combined Nonsimultaneous Radiation Therapy and Chemotherapy With 5-FU, Doxorubicin, and Mitomycin For Residual Localized Gastric Adenocarcinoma: A Southwest Oncology Group Pilot Study[1,2]

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Efforts to control gross or microscopic residual gastric adenocarcinoma with radiation therapy and/or chemotherapy have met with limited success (1–5). Excessive toxicity and mortality during the first several months of treatment have been described for a regimen combining simultaneous 5-FU at a dose of 500 mg/m^2 on Days 1, 2, and 3 of each of two 2500-rad sessions of radiation therapy, for which midplane field size area was restricted to 400 cm^2. Continued analysis and reporting of the results of that regimen, however, now show a distinct, late survival advantage for patients treated with these combined modalities when compared to an equivalent group of patients randomized to treatment with 5-FU and semustine without radiation (5).

The impressive early results reported for the combination of 5-FU, doxorubicin, and mitomycin (5-FAM) in patients with advanced gastric cancer (6,7) prompted us to combine radiation therapy with these drugs in patients with well-defined residual gross or microscopic gastric cancer after exploratory surgery. We modified 5-FAM for this study by deleting the dose of 5-FU scheduled on Days 8 and 35 and by reducing the remaining doses of 5-FU to 500 mg/m^2 and the dose of mitomycin on Day 1 to 8 mg/m^2. The dose of doxorubicin was not reduced to maximize its interaction with radiation. Radiation was planned by a split course of three sessions of 1500 rads each and was limited to 225 cm^2 in the frontal plane to minimize gastrointestinal intol erance. Supradiaphragmatic structures were excluded from the radiation fields to minimize risk of cardiac and pulmonary toxic effects; thus, patients with residual disease in the mediastinum were excluded.

These considerations resulted in a combined modality regimen with acceptable toxicity as reported below.

PATIENTS AND METHODS

Adult patients with histologically proven residual gastric cancer were entered after obtaining informed consent. Eligibility requirements included: wbc count $\geq$ 4000/μl, platelet count $\geq$ 100,000/μl, serum bilirubin $\leq$ 2 mg/dl, creatinine clearance $\geq$ 60 ml/minute, no history of congestive heart failure, no prior radiotherapy or chemotherapy, no distant metastatic disease, and all known residual disease restricted to a 225-cm^2 contiguous area. Treatment was begun between 4 and 8 weeks after surgery. Residual disease sites were to be identified by placement of metallic clips during surgery or to be defined by ultrasound or computerized tomography. Patients with either gross or microscopic residual disease were accepted. Chemotherapy dose schedules were: 5-FU, 500 mg/m^2 iv on Days 1, 29, 57, and 85; doxorubicin, 30 mg/m^2 iv on Days 1, 29, 57, and 85; and mitomycin, 8 mg/m^2 iv on Days 1 and 57.

Radiation therapy consisted of three sessions of 1500 rads each (daily dose, 180–200 rads in a minimum of seven daily fractions in 9 elapsed days and a maximum of nine daily fractions in 13 elapsed days) which were prescribed to begin on Days 8, 36, and 64. Each radiation session was thus begun 1 week after each of the first three courses of 5-FAM (mitomycin was deleted from the second cycle). The total planned midplane dose was 4500 rads in 10 weeks. Use of supervoltage equipment of at least 2 mev or cobalt 60 sources was specified. Maximum portal size was 225 cm^2. Supradiaphragmatic structures were excluded from any portal; posterior kidney blocks were used and not > 50% of either kidney was included in the treatment volume; maximum liver volume included was 33%; and the spinal cord dose was

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limited to 4000 rads. Most patients were treated by shaped, opposed anterior posterior portals, but multiple-field techniques were allowed and recommended.

After completion of the combined radiation and chemotherapy sequence described above, patients continued to receive the same chemotherapy regimen for an additional four courses at 56-day intervals unless disease progression was observed.

Chemotherapy was delayed if the wbc and platelet counts were < 3000 and/or 75,000/μl, respectively, until recovery to at least those levels. Day-29 (of each course of 56 days) doses of 5-FU and doxorubicin were reduced by 25%, 33%, and 50% for wbc count nadirs of 3000–3999, 1000–2999, and < 1000/mm^3, respectively. Subsequent courses were adjusted as follows based on the previous course's toxicity (table 1). Radiation was delayed when necessary for a wbc count < 3000/mm^3, a platelet count < 100,000/mm^3, or for significant radiation enteritis, and was stopped if disease progression was documented.

Patients were evaluated prior to treatment with history and physical examination, cbc, platelet count, BUN, creatinine, bilirubin, SGOT, alkaline phosphatase, CEA, chest x-ray, upper gastrointestinal series, and liver scan; the latter three were repeated every 4 months, the chemistries every 2 months, and the cbc and platelet counts every 2 weeks (twice weekly during radiation therapy sessions).

All patients have been followed until death or for at least 3 years. Patient entry began in September 1978 and ended in October 1979. The study was amended in January 1979 to allow entry of patients with first local recurrence, provided that they had received no prior radiation or drug treatment; only one such patient (No. 4) was entered.

TABLE 1.—Adjustment of doses based on toxicity

Wbc count nadir (cells × 10^3/μl)		Platelet count nadir (cells × 10^3/μl)	Dose change for all drugs
> 4.0		100	+10%
3.0–3.9	*and/or*	75–99	No change
2.0–2.9		50–74	−10%
1.0–1.9		25–49	−33%
< 1.0		< 25	−50%

TABLE 2.—Patient characteristics, patterns of relapse, and survival

Patient No.	Type of surgery	Residual sites	Gross or microscopic residual disease	% weight change*	Days to disease progression	Survival in days	Site of 1st recurrence or relapse
1	Biopsy	Stomach and celiac nodes	Gross	+0.9	137	492	Persistent in stomach
2	Total gastrectomy	Nodes and pleura	Microscopic (suspected in pleura also)	−11.6	57	156	Pleura
3	Biopsy	Gastric stump	Gross	−3.8	176	359	Meninges
4	Partial gastrectomy and pancreatectomy	Pancreas (200 days after initial surgery)	Gross	−7.2	436	525	Subcutaneous, resected, then skin and liver
5	Esophagogastrectomy	Celiac nodes and resection line	Gross and microscopic	−6.2	97	109	Lung, pleura, and liver
6	Partial gastrectomy	Nodes	Gross	−4.5	139	213	Pleura and peritoneum (Day 139), then local + bone
7	Partial gastrectomy and transverse colectomy	Nodes and resection margin	Microscopic	−1.4	351	425	Liver
8	Biopsy	Gastroesophageal junction and nodes	Nodes	+2.1	169	409	Supraclavicular node and local
9	Partial gastrectomy	Nodes	Gross	−1.3	270	486	Local recurrence
10	Partial gastrectomy	Proximal resection	Microscopic	−7.1	291	333	Local recurrence
11	Esophagogastrectomy, splenectomy, and partial pancreatectomy	Aorta	Gross	−15.9	421	434	Pelvic peritoneum
12	Partial gastrectomy	Nodes	Microscopic	−3.0	1155+	1155+	—

*Observed weight change during and 6 wks after radiation therapy.

RESULTS

Twelve patients were registered and all are reported herein. Patient characteristics are shown in table 2. Patient 2 had pleural metastases (in retrospect) at the time treatment was started. Patient 4 was the only patient treated for a first recurrence. Patient 12 refused further chemotherapy after the first day but completed the prescribed radiation course on schedule; she is the only relapse-free patient of this series. The other 11 patients have relapsed or had disease progression in the sites and times shown in table 2.

Total radiation doses in the 11 patients completing three planned courses ranged from 4436 to 4737 rads. Significant radiation delays (> 2 weeks) were necessary only for Patient 11 who had prolonged, severe courses of radiation enteritis during each session of radiation therapy. Weight loss and debilitation were corrected by iv hyperalimentation. Minor radiation delays (up to 2 weeks) were necessary in Patient 10 due to leukopenia and thrombocytopenia. Patient 5 completed the first two sessions of radiation; the third patient was terminated because of abdominal pain and vomiting which ultimately proved to be due to disease progression with diffuse peritoneal seeding.

All patients experienced myelosuppression that is summarized in table 3, but only Patient 7 experienced an episode of leukopenia of < 1000 cells/μl. There is no clear trend toward cumulative myelotoxicity during the combined modalities sessions. The dose adjustment scheme described above provides a reasonable basis for continued therapy upon recovery from the prior course's acute toxic effects. No treatment-related deaths, sepsis, or bleeding occurred. Patient 8 developed a histologically benign gastric ulcer outside of the radiation port during the third radiation session; it healed without complications with medical management.

Alopecia as well as varying degrees of vomiting occurred in all patients with each cycle of chemotherapy. Only Patient 12 refused further chemotherapy on this basis.

Percentage of body weight change during the radiation-chemotherapy sequence period ranged from gains of 1% to 2% (two patients) to losses of > 10% (two patients). The latter two patients had extensive resections (total gastrectomy, esophagogastrectomy, and partial pancreatectomy), whereas the two patients who gained weight had had biopsies. Median percent weight change during the radiation period for the three biopsied-only patients was +0.9% (range, −3.8% to +2.1%), for the seven patients with partial gastrectomy plus other resection was −4.5% (range, −1.3% to +7.2%), and for the two patients with total gastrectomy ± other resection was 11.6% and 15.9%. This suggests that more extensive gastric resections produce poorer tolerance of vigorous combined modalities therapy and that such patients should be considered for early or prophylactic parenteral alimentation.

Median time to relapse or disease progression was 224 days (range, 57–1155+); median survival time was 417 days (range, 109–1155+). As mentioned, only one patient enjoys relapse-free status and survival at the time of this report. Numbers of patients in the gross versus microscopic residual disease categories are too small for meaningful differences to be detected. Relapse-free intervals for the three patients with only microscopic residual disease were 291, 351, and 1155+ days (median, 351), while for patients with gross residual disease relapses occurred from 97 to 421 days (median, 173). Median survival times for these same groups

TABLE 3.—Gastric cancer treated with radiation therapy and 5-FAM chemotherapy: myelosuppression during combined modalities treatment (11 patients)*

	Cycle—†			
	1 (5-FAM + RT)‡	2 (5-FA + RT)§	3 (5-FAM + RT)¶	4 (5-FA)**
Wbc count nadir (× 10^3 cells/μl)				
Median	2.8	3.2	3.2	2.6
Range	1.7–4.6	1.8–5.3	0.7–3.6	1.7–5.6
No. of patients with wbc counts ≥ 4.0	2	1	0	2
Platelet count nadir (× 10^3 cells/μl)				
Median	191	180	150	325
Range	75–360	150–295	61–295	89–330

*Patient 12 refused chemotherapy and blood cell counts after 1st day of chemotherapy, but received prescribed radiation.
†RT = radiation therapy (1500 rads); 5-FA = 5-FU + doxorubicin.
‡Given on Days 1–28.
§Given on Days 29–56.
¶Given on Days 57–85.
**Given on Days 86–114.

were 425 and 422 days, respectively, with the only relapse-free survivor occurring in the microscopic residual disease group. Local freedom from relapse or disease progression within the residual tumor sites was achieved in two of three patients with microscopic residual tumor (excluding Patient 2 with pretreatment pleural disease) and in three of eight with gross residual tumor. Thus, five of 11 patients had freedom from local relapse or disease progression during these periods of survival.

DISCUSSION

This pilot study has defined a combined radiation-chemotherapy regimen producing acceptable toxicity for patients with localized, residual gastric adenocarcinoma who have been staged surgically with or without resection. Hematologic toxicity was noted in all patients during their treatment course. Leukopenia was more common and of greater degree than thrombocytopenia in all patients. No treatment-related deaths occurred. More vigorous chemotherapy would probably be intolerable without supportive measures for prolonged, substantial leukopenia.

Patients with total gastrectomies appear predisposed to excessive weight loss and should receive appropriate nutritional support either prophylactically or very quickly to avoid debilitation from radiation enteritis.

Excessive early toxicity and mortality noted with simultaneous radiation and 5-FU by the Gastrointestinal Tumor Study Group (GITSG) in a similar group of patients (5) were not observed in this pilot study. Median survival of this group (14 months) is comparable to that reported by Thirwell et al (4), and is slightly better than that of the GITSG's combined modalities group (9 months) (5) and the results reported by the Mayo Clinic (7 months) (1,2). All three of these combined modalities patient groups have small numbers of long-term survivors. The limited patient accrual of this pilot study precludes a definitive statement regarding ultimate efficacy of this treatment, much less any meaningful comparison with other reported trials. Further evaluation of this regimen is warranted.

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Daunorubicin, Vincristine, and Prednisone for Remission Induction in Patients With Acute Lymphoblastic Leukemia in Relapse[1]

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Patients with recurrent acute lymphoblastic leukemia were treated with daunorubicin (40 mg/m²/week × 4), vincristine (1.5 mg/m²/week × 4), and prednisone (40 mg/m²/day × 28). All of the patients had been treated with the same combination during the first induction treatment. Of 266 patients (221 children and 45 adults) treated in first relapse, 141 (53%) achieved complete remission (CR; 55% of the children and 44% of the adults). Of 61 patients who were re-treated with the same combination after the second relapse, 14 (23%) achieved CR. The difference between second and third CR was statistically significant ($P < 0.0005$). The median durations of second and third CR were 8 and 6 weeks, respectively. No significant difference was observed when the duration of CR was compared with the initial wbc count, age at diagnosis, or duration of first CR. [Cancer Treat Rep 67:425–427, 1983]

Vincristine, daunorubicin, and prednisone form an effective induction combination for previously untreated patients with acute lymphoblastic leukemia (ALL). The complete remission (CR) rate reported is between 90% and 95% (1,2). Several combinations with two, three, and four drugs have been explored for second and subsequent relapses. The CR rates varied from 40% to 90% (3–9).

In this report we present the experience of the Argentine Group for the Treatment of Acute Leukemia with daunorubicin, vincristine, and prednisone in the treatment of single and multiple consecutive relapses occurring during maintenance chemotherapy. We also evaluated the prognostic factor determining the rate of CR and the duration of subsequent remissions.

PATIENTS AND METHODS

The patients in this study had been previously admitted to first-line protocols 10-ALL-72 (2) and 1-ALL-76 and 1-ALL-79 (10); these were three consecutive protocols carried out from October 1972 to December 1981 for previously untreated ALL patients. Patients received daunorubicin, vincristine, and prednisone as first induction treatment. Maintenance therapy consisted of 6-mercaptopurine and methotrexate, with periodic reinductions with one dose of vincristine and 1 week of prednisone. Daunorubicin was used only during induction. Patients in protocol 10-ALL-72 received cranial irradiation and intrathecal methotrexate as CNS prophylaxis, while patients admitted to protocols 1-ALL-76 and 1-ALL-79 received only intrathecal methotrexate-dexamethasone during induction and maintenance.

A total of 266 patients (221 children ≤ 15 years of age and 45 adults) who relapsed in bone marrow during maintenance chemotherapy were included in this evaluation. The ages of the patients ranged from 2 to 51 years, with a median age of 10.3 years.

The remission induction regimen was: vincristine, 1.5 mg/m² iv weekly × 4; daunorubicin, 40 mg/m² iv weekly

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TABLE 1.—2nd and 3rd CR in ALL treated with daunorubicin, vincristine, and prednisone, according to age

Age (yrs)	No. of patients	No. (%) of patients with— CR	PR	No response
		First relapse		
≤ 15	221	121 (55)*	15	85
> 15	45	20 (44)	5	20
		Second relapse		
≤ 15	56	13 (23)*	10	33
> 15	5	1 (20)	1	3

*$P < 0.0005$.

× 4; and prednisone, 40 mg/m² orally daily × 28. For remission maintenance most of the patients were treated again with 6-mercaptopurine and prednisone, with pulses of vincristine and prednisone sequentially with cytarabine and cyclophosphamide.

Sixty-one patients (56 children and five adults) received this combination in their second bone marrow relapse.

Patients who had received therapy for > 7 days were considered adequately treated and were included for analysis.

CR was defined as having < 5% blast cells in a normocellular bone marrow. When the bone marrow had > 5% but < 25% blast cells, the patient was considered to be in partial remission (PR). Bone marrow relapse was defined as bone marrow that contained > 25% blast cells. Life tables and the log-rank test were employed to compare duration of CR among groups.

RESULTS

Of the 266 patients treated with daunorubicin, vincristine, and prednisone for a first bone marrow relapse, 141 (53%) achieved CR, 20 achieved PR, and 105 had no response or early death. The CR rate was higher in children (55%) than in adults (44%); however, this difference was not significant (table 1).

Of 141 patients who achieved CR and had a second bone marrow relapse, 61 were re-treated with the same combination; 14 (23%) achieved a third CR, 11 achieved PR, and 36 failed to respond. The difference in the CR rates between the first and second relapse is highly significant ($P < 0.0005$).

Duration of complete remission.—The median duration of the second CR was 8 weeks, with 25% and 11% of the patients still in CR at 4 and 8 months, respectively. The median duration of CR for patients in third CR was 6 weeks. Those who had a longer first CR had a longer second CR. The median duration of CR was 8 weeks for those who relapsed before 12 months, 9 weeks for those who relapsed between 12 and 24 months, and 11 weeks for those who relapsed after 24 months from achieving their first CR. The difference was not statistically significant.

The median durations of CR according to wbc count at diagnosis (≤ or > 50,000/mm³) were 9 and 6 weeks, respectively, with 18% and 9% of the patients remaining in CR at 6 months. This difference was not significant. Patients ≤ or > 15 years of age who achieved a second CR had similar median durations of CR: 9 and 8 months, respectively.

DISCUSSION

The CR rate with vincristine, daunorubicin, and prednisone in previously untreated patients with ALL ranged from 89% to 95% (1,2). This rate was slightly higher than that with vincristine and prednisone alone in the overall group and standard-risk children, without being statistically significant. However, in high-risk children and adults with ALL the rate of CR was statistically higher with the three-drug combination than with vincristine and prednisone alone (10).

The present study explored the remission rate obtained with daunorubicin, vincristine, and prednisone in patients in relapse who had been treated during the first induction with the same three-drug combination and in the same schedule and dosage. The CR rates were 53% after the first bone marrow relapse and 23% after the second bone marrow relapse. Children had a higher CR rate than adults. The duration of second CR was dramatically short, ie, 2 months.

Recently, there have been reports of large series of patients in relapse who have been treated in institutions of cooperative groups with two-, three-, or four-drug combinations. The Cancer and Leukemia Group B (6) reported 227 patients with recurrent ALL treated with various combinations of vincristine, prednisone, cyclophosphamide, and L-asparaginase. Of patients receiving L-asparaginase, 73% achieved CR, while only 46% of those not receiving L-asparaginase achieved CR ($P < 0.05$). In a consecutive protocol, the addition of cy-

clophosphamide to vincristine, prednisone, and L-asparaginase did not improve the remission induction rate, which remained around 72%.

Various combinations of cytarabine, cyclophosphamide, vincristine, prednisone, carmustine, or lomustine failed to maintain remission duration for >2–3 months (6).

The Children's Cancer Study Group (3) reports the results of 413 patients reinduced to remission with daily 6-mercaptopurine and different doses of L-asparaginase given im three times weekly. All of the patients had experienced several previous relapses. L-Asparaginase was found to be effective in reinducing remissions at the following rates: 9.5% for 300 IU/m^2, 35% for 3000 IU/m^2, 53% for 6000 IU/m^2, and 62% for 12,000 IU/m^2.

More recently, Reaman et al (7) from the Pediatric Oncology Branch of the National Cancer Institute (Bethesda, MD) published a study in which ALL patients in relapse were treated with a four-drug reinduction regimen consisting of L-asparaginase, vincristine, daunorubicin, and prednisone. CR was achieved in 114 of 125 (91%) treatment courses. CR rates for second and third reinduction attempts were 89% and 90%, respectively.

Another drug combination reported is that employed by Rivera et al (9) with teniposide and cytarabine. Nine of 33 children with ALL in relapse achieved CR.

The duration of second CR was very short (2–3 months) in most of the published studies using different intensification and maintenance combinations (6,8,9). The longest CR (8 months) was reported by Reaman et al (7) using four drugs as induction.

The major prognostic factor to estimate the rate and duration of CR was the number of previous relapses. The effects of initial wbc count, duration of first CR, and age have little or no prognostic value in measuring the duration of the subsequent hematologic remission (4,11).

Sather et al (12) from the Children's Cancer Study Group found that major prognostic factors at diagnosis, such as wbc count, age, and sex, gradually lose prognostic importance. After 15 months in CR, the prognostic value of age and sex is negligible, while initial wbc count loses its prognostic value at 24 months.

We can conclude that in patients with ALL in first and second relapse who were treated during the first induction with daunorubicin, vincristine, and prednisone, the same combination produced low CR rates and short CR. According to the study of Reaman et al (7), the addition of high doses of L-asparaginase probably can significantly increase the rate and duration of CR.

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Microangiopathic Hemolytic Anemia, Renal Failure, and Noncardiogenic Pulmonary Edema: A Chemotherapy-Induced Syndrome[1]

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Following gastrectomy for locally advanced adenocarcinomas, three patients developed microangiopathic hemolytic anemia and renal failure shortly after completing courses of adjuvant chemotherapy with mitomycin and 5-FU. These complications progressed despite cessation of chemotherapy, and all three patients died of noncardiogenic pulmonary edema precipitated in two cases by blood transfusions. At autopsy, two patients had no residual carcinoma and all had a diffuse microangiopathy involving mainly the kidneys and lungs. There was intimal hyperplasia of many arterioles sometimes associated with complete occlusion of the lumen, prominent nuclear atypia in many capillary cells, and numerous capillary fibrin thrombi. Direct immunofluorescence studies revealed extensive fibrinogen-fibrin deposits in the vascular lesions. Chemotherapy-induced microangiopathic hemolytic anemia and renal failure may predispose patients to fatal episodes of noncardiogenic pulmonary edema that can be triggered by blood transfusions. [Cancer Treat Rep 67:429–434, 1983]

In recent years, chemotherapy has increasingly been given as adjuvant therapy after primary treatment of certain neoplasms (1,2). Consequently, drug-related complications may occur in a setting where the patient does not have any residual tumor. This paper will describe three patients who, after receiving 5-FU and mitomycin (MMC) following resection of locally advanced gastric carcinomas, died of noncardiogenic pulmonary edema complicating microangiopathic hemolytic anemia (MAHA) and renal failure.

These three patients were the first patients entered on a gastric carcinoma adjuvant chemotherapy protocol at our institution. The treatment consisted of 5 mg of MMC and 500 mg of 5-FU given iv monthly on 4 consecutive days. No other patients were entered on the protocol, which was terminated after the occurrence of these complications. Two of the patients had no residual tumor at autopsy, and two of the fatal pulmonary episodes were precipitated by blood transfusions given to treat the underlying MAHA. Since 5-FU and MMC are increasingly used in combination chemotherapy regimens with doxorubicin (3), both for advanced carcinomas and experimentally as adjuvant therapy, it is important for clinicians to be aware of these complications.

CASE REPORTS

Patient 1

A 58-year-old white woman underwent a radical gastrectomy for a linitis plastica carcinoma of the antrum with positive perigastric nodes. Chest x-ray and renal function were normal. The patient received a total of 58.8 mg/m^2 of MMC and 5.88 g/m^2 of 5-FU as adjuvant chemotherapy over the following 8 months. At the end of that period, the patient's hemoglobin (Hb) dropped to 7.5 g/100 ml, with a wbc count of 3800/mm^3, a platelet count of 52,000/mm^3, and schistocytes noted on the peripheral smear. BUN had increased to 30 mg/dl, and the creatinine had decreased to 1.4 mg/dl. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were normal, and serum haptoglobin was < 50 mg/100 ml (normal, 50–220). Six units of packed rbcs

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FIGURE 1.—Large unfolded atypical nucleus in an alveolar wall cell. Uranyl acetate and lead citrate stain, × 7000.

were transfused at intervals over the following month, without improvement in the anemia. BUN rose to 52 mg/dl, creatinine increased to 2.6 mg/dl, and urinalysis revealed 3+ proteinuria and microscopic hematuria with a strongly positive urinary hemosiderin. Fibrinogen level was 145 mg/100 ml (normal, 200–400), with the antithrombin III at 21.5 mg/100 ml (normal, 20–40). PT and APTT were normal; fibrin degradation products were absent, and protamine sulfate and ethanol gel reactions were negative. Bone marrow aspiration showed decreased megakaryocytes, but marrow specimens were otherwise unremarkable. Rectal bleeding prompted hospital admission. Rectoscopy and barium enema were normal, and the patient stopped bleeding spontaneously after receiving 4 units of whole blood.

The patient was readmitted 1 month later, after an episode of epistaxis with persistent severe anemia. Laboratory tests were unchanged from the previous admission. During a transfusion of packed rbcs shortly after admission, the patient rapidly developed pulmonary edema. The transfused blood had not been mismatched. She died 4 days later after having refractory pulmonary edema with severe hypoxemia and metabolic acidosis unresponsive to treatment.

At autopsy, there was no evidence of recurrence of the gastric carcinoma. The heart was normal, but the lungs were severely congested with hemorrhagic alveolar edema on microscopic examination. Many endothelial and alveolar cells containing dark, large, and atypical nuclei were found (fig 1), and numerous pulmonary and bronchial arterioles showed intimal hyperplasia sometimes associated with almost complete occlusion of the vascular lumen. Microscopic examination of the kidneys revealed many glomeruli with fibrin thrombi in the capillary loops, basement membrane thickening, and mesangiolytic changes (fig 2). Many atypical, sometimes vacuolated nuclei were seen in cells of the glomerular tufts as well as in tubular epithelial cells. Fibrin thrombi and fibrinoid necrosis or intimal hyperplasia were also noted in the renal arterioles. Similar but less severe microangiopathic changes were also observed in other systemic vessels. Direct immunofluorescence revealed extensive fibrinogen-fibrin deposits in some glomerular vessels, while immunoglobulins or complement products were not detected (fig 3). Electron microscopy revealed the presence of floccular material, sometimes associated with fibrin strands, in glomerular capillary lumens.

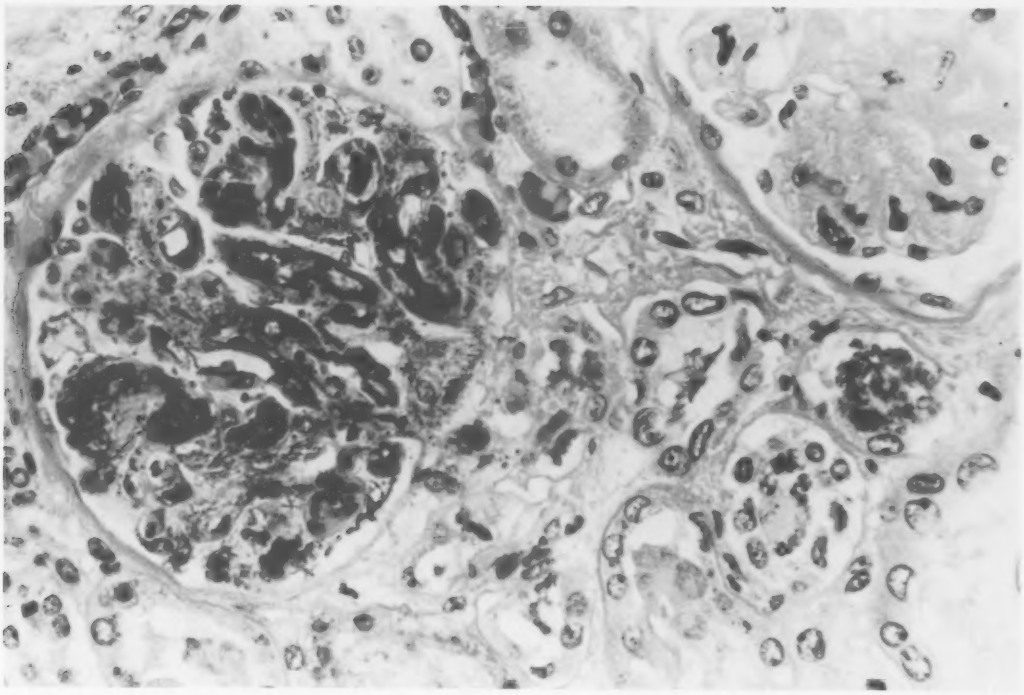

FIGURE 2.—Kidney histology from Patient 1 showing a glomerulus with extensive fibrin thrombi in the capillary loops. The other glomerulus at the upper right has mesangiolytic changes and irregular nuclei. Mallory fibrin stain, × 400.

Patient 2

A 56-year-old white woman underwent a radical gastrectomy for a linitis plastica carcinoma with positive perigastric nodes. Over the next 4 months, she received a total of 59.3 mg/m^2 of MMC and 5.93 g/m^2 of 5-FU as adjuvant chemotherapy. One month after stopping chemotherapy, the patient presented with severe weakness, an Hb of 4.5 g/100 ml, a wbc count of 4800/mm^3, a platelet count of 39,000/mm^3, and a blood smear showing schistocytes and marked anisopoikilocytosis. Bone marrow aspiration specimens were unremarkable except for slightly decreased megakaryocytes. The haptoglobin level was < 50 mg/100 ml, and urinary hemosiderin was negative. The fibrinogen level was 420 mg/100 ml, the antithrombin III was 25.2 mg/100 ml, and the PT and APTT were normal. There were no detectable fibrin degradation products in the plasma, ethanol gel was negative, and the protamine sulfate reaction was slightly positive. BUN had increased to 41 mg/dl, and serum creatinine had increased to 1.8 mg/dl, with a clearance of 18 ml/minute. Urinalysis revealed 2+ proteinuria and microscopic hematuria. Four units of packed rbcs were given slowly over 2 days. The patient became slightly dyspneic but responded promptly to administration of furosemide. Treatment with heparin (1000 units iv every 4 hours for 6 days) was without success. Two weeks after admission and 8 hours after receiving a single unit of packed rbcs, the patient rapidly developed pulmonary edema, with severe hypoxemia and metabolic acidosis. She was unresponsive to treatment and died 8 hours later.

At autopsy, there was no evidence of abdominal recurrence of the gastric adenocarcinoma, but unsuspected microscopic bone metastases were discovered. The heart was normal. The findings on microscopic examination, immunofluorescence studies, and electron microscopy were identical to those reported for the first patient, except that numerous fibrin thrombi were identified in the pulmonary microcirculation (fig 4).

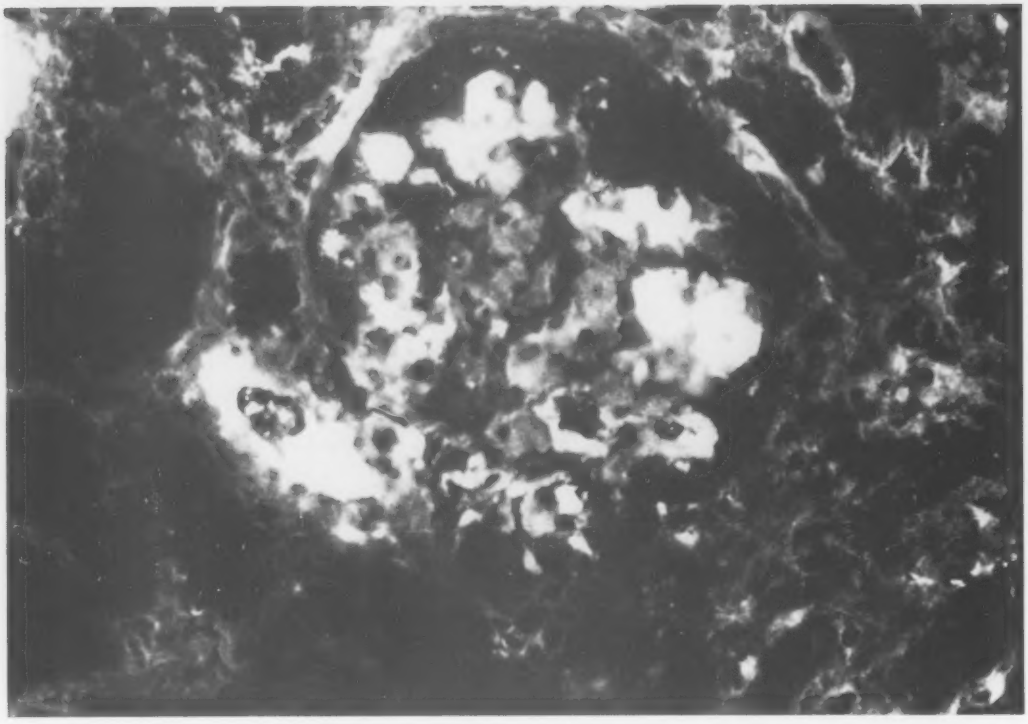

FIGURE 3.—Section of the same kidney showing conspicuous fibrin deposits in the tuft and adjacent arteriole. Frozen section incubated with fluorescein isothiocyanate (FITC)-labeled serum against human fibrinogen-fibrin-related antigens (FRA), × 400.

Patient 3

A 37-year-old woman underwent a gastrectomy for adenocarcinoma with positive perigastric nodes. She was given adjuvant chemotherapy with MMC and 5-FU over a period of 7 months to cumulative doses of 74.8 mg/m^2 (MMC) and 8.06 g/m^2 (5-FU). Therapy was stopped because of mild pancytopenia. Four months after stopping chemotherapy, the patient was admitted to the hospital with severe weakness; laboratory investigation revealed an Hb of 8.0/100 ml, a wbc count of 2600/mm^3, a platelet count of 171,000/mm^3, and schistocytes on the peripheral smear. Serum creatinine had risen to 2.2 mg/dl, and 2+ proteinuria was noted on urinalysis. No blood transfusions were given. One week following admission the patient rapidly developed severe pulmonary edema without any obvious precipitating factor. A Swann-Ganz catheter recorded the following pressures: mean pulmonary arterial pressure, 19 mm Hg; pulmonary wedge pressure, 8 mm Hg; and cardiac output, 4.6 L/m^2. The patient died 2 days later despite intensive therapy. At autopsy there was no evidence of adenocarcinoma, and no cardiovascular abnormalities to explain the pulmonary edema were found. Renal and pulmonary lesions were similar to those seen in the first two cases. The microangiopathic changes observed in several systemic vessels were particularly severe in this case.

DISCUSSION

The three patients described died of noncardiogenic pulmonary edema occurring after the appearance of MAHA and renal failure. They were the first patients to be entered on a gastric carcinoma adjuvant chemotherapy protocol at our institution. We had not observed these complications in other patients with advanced gastrointestinal malignancies treated with 5-FU and MMC either alone or in combination with doxorubicin (3), suggesting an enhanced toxic potential for the chemotherapy regimen used in this adjuvant setting.

During the past 3 years, at least 32 cases of chemotherapy-related MAHA and renal failure have been de-

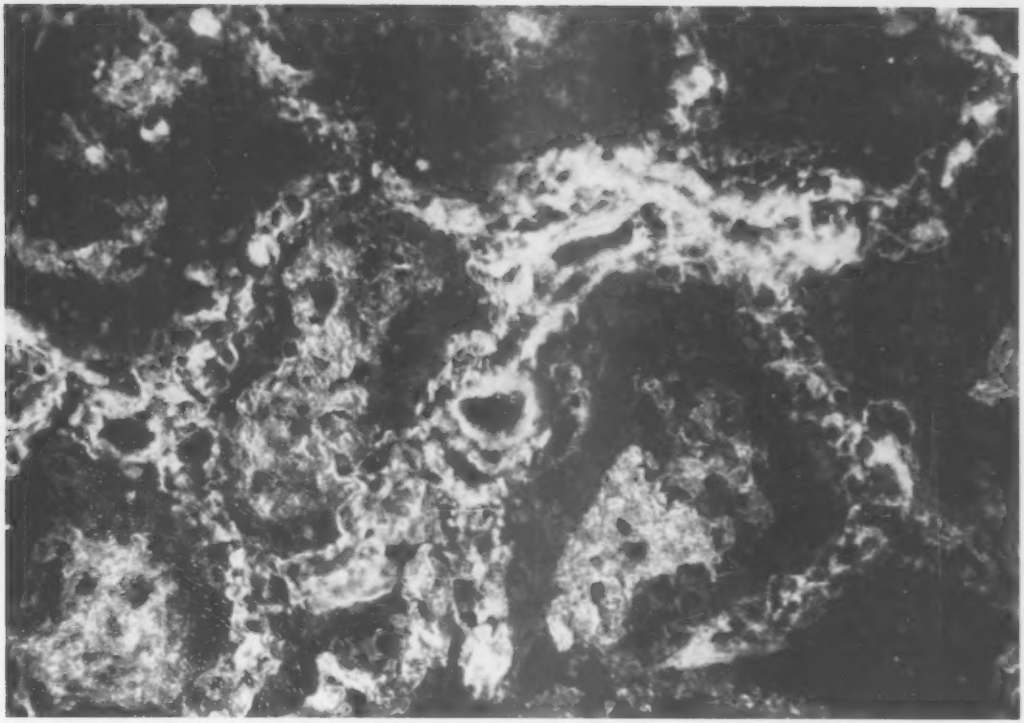

FIGURE 4.—Frozen section of the lung from Patient 2. Fibrin deposits were present in vessel walls, in alveolar capillaries, and within the edematous fluid in alveolar lumen. Section incubated with FITC-labeled anti-FRA serum, × 250.

scribed in the literature (4–17). All but five patients who developed this syndrome were either receiving or had recently received chemotherapy regimens containing MMC. Attempts at controlling these complications with heparin, dipyridamole, steroids, or plasma infusions were unsuccessful except in three patients in whom the complications were controlled by plasmapheresis (6,14,17). The renal histopathology described in our cases and in other reports (5,12,13) closely resembles that of the lesions seen in the hemolytic-uremic syndrome and thrombocytic thrombocytopenic purpura (18). Our cases are clinically distinct from these two disorders, however, since pulmonary complications are not a major organ involvement in hemolytic-uremic syndrome or thrombocytic thrombocytopenic purpura. Although volume overload can lead to congestive heart failure in azotemic patients, the pulmonary abnormalities were likely due to increased capillary permeability in these cases, in view of the normal hearts, the abnormal pulmonary vasculature and markedly hemorrhagic alveolar edema seen in the three autopsies, and the normal pulmonary wedge pressures measured in the third patient. Pulmonary toxicity secondary to MMC has been described in the literature (19–21) and has coexisted with MAHA and renal failure in one reported patient (10). The reported pulmonary lesions, however, consisted mostly of interstitial fibrosis without any vascular or nuclear atypias and were not usually associated with renal or hematologic abnormalities, suggesting that they resulted from a different pathogenic mechanism.

The cause of this syndrome of complications is unknown, although it is difficult to implicate the neoplasms as causative factors in many cases, since two of our patients and at least five other reported patients had no detectable tumor at autopsy (7,11,13,15) and one patient survived the complications and had no clinical tumor recurrence after stopping chemotherapy (6). Indeed, MAHA associated with malignancy usually occurs in patients with advanced neoplasms and is not associated with the renal or pulmonary abnormalities seen in our patients (22). The complications were probably chemotherapy-related since they occurred during or shortly after the administration of antineoplastic agents and were also associated in our patients and in some

case reports (5,12,13) with prominent nuclear atypia which are often a feature of toxic lesions induced by alkylating agents (23).

An intriguing clinical observation was that transfusions triggered the episodes of pulmonary edema in two of our patients and in two reported cases (8,14). Furthermore, the anemia failed to improve after transfusions in our first patient. Agnelli et al (24) recently reported that intravascular clotting was activated after rbc transfusions in a patient with pancreatic carcinoma treated with MMC, 5-FU, and doxorubicin (FAM regimen) and that this activation could be minimized by the administration of heparin (2500 IU iv) prior to a subsequent transfusion. Further intravascular coagulation in patients with already widespread fibrin microthrombi could worsen existing organ dysfunctions. The recent observations that a platelet-associated IgG was elevated in a case of chemotherapy-related MAHA and renal failure (14) and formed immune complexes in another case (17) also suggest that immunologic mechanisms could have been involved in the episodes of noncardiogenic pulmonary edema following transfusions.

MAHA and renal failure complicating cancer therapy can predispose patients to episodes of noncardiogenic pulmonary edema, especially after blood transfusions.

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Phase I Trial of Mitoxantrone by 24-Hour Continuous Infusion[1]

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Mitoxantrone (DAD) is a new agent which intercalates into DNA. Preclinical studies have demonstrated activity equal to or greater than that of doxorubicin in all tumor systems tested. In this phase I clinical trial, the schedule of drug administration consisted of a 24-hour continuous iv infusion repeated at 21-day intervals. Twenty-nine patients received a total of 66 courses over a dose range of 4–15 mg/m². The dose-limiting toxic effect was leukopenia, with a wbc count nadir on Day 12 and resolution prior to Day 21. Other toxic effects were thrombocytopenia, mild phlebitis, and blue discoloration of veins. Objective tumor responses were seen in a patient with adenocarcinoma of the breast and in another patient with clear cell carcinoma of the vagina. An additional six patients with acute leukemia were treated at a dose of 12 mg/m²; a decrease in peripheral blast count was observed in four of these six patients. The toxicity of DAD by 24-hour iv infusion was similar to that previously reported for iv bolus administration. We recommend phase II evaluation of DAD at a dose of 12 mg/m² by single iv injection at 21-day intervals. Patients with acute leukemia should be evaluated at higher dose levels. [Cancer Treat Rep 67:435–438, 1983]

Mitoxantrone (DAD) is one of the more active members of a series of substituted aminoalkylaminoanthraquinones. These agents contain an electron-rich planar ring structure which is an essential feature for intercalation (1). Most of these substituted anthraquinones are strong DNA binding agents which produce elevations in DNA melting temperatures comparable to, or greater than, those produced by the anthracycline antibiotics. In vivo antitumor activity, however, does not correlate well with in vitro measures of DNA binding capability (2); thus, an effect other than, or in addition to, DNA intercalation may account for the antitumor activity of these drugs.

In preclinical studies, DAD produced a > 200% increase in lifespan and a high frequency of cures in P388 and L1210 murine leukemias. It also produced a significant increase in survival and a high frequency of cures in B16 melanoma and colon carcinoma 26. In every tumor system tested, the efficacy of DAD was comparable to, or greater than, that of doxorubicin. In a doxorubicin-resistant strain of P388 leukemia, the activity of DAD, although reduced, was still significant (2).

In animal toxicology studies, the beagle dog was the most sensitive animal species, with a toxic dose low of 3.86 mg/m² in a single dose schedule and 0.515 mg/m²/day in a 5-day dose schedule. The major toxic effects observed were hematologic dose-related reversible leukopenia, anemia, and thrombocytopenia in association with bone marrow hypoplasia (3). Preliminary pharmacokinetic data in dogs indicated rapid plasma clearance of drug after iv bolus administration (3). This prompted us to utilize a 24-hour continuous infusion of DAD to ensure prolonged tissue exposure. The purpose of this study was to determine the dose-limiting toxicity and the maximum tolerated dose of DAD given as a 24-hour iv infusion.

MATERIALS AND METHODS

Patient selection.—All patients had histologic confirmation of malignant disease that was refractory to conventional therapy or for which there was no therapy of

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proven clinical benefit. All patients had normal hematologic, renal, hepatic, and cardiac functions, and all had an estimated survival of > 2 months. Objectively measurable disease was not required. Written informed consent in accordance with Federal and institutional policies was obtained from all patients prior to entry into this study.

Treatment plan.—DAD was supplied by the Investigational Drug Branch, National Cancer Institute (Bethesda, MD), as a sterile solution in 10-ml ampules, with a drug concentration of 0.5 mg of free base/ml of solution. Inert ingredients were (wt/vol) sodium metabisulfite (0.200%), sodium chloride (0.800%), and water for injection. The calculated DAD dose was added to 1000 ml of normal saline and infused iv over a period of 24 hours with a constant infusion pump (IMED, San Diego, CA). The single 24-hour infusion was repeated every 21 days.

Starting dose.—In a recently completed phase I study of DAD given by rapid iv infusion every 4 weeks, dose-limiting myelosuppression occurred at 12 mg/m^2 (4). Based on this information, we selected 4 mg/m^2 by 24-hour continuous infusion as a safe starting dose for our phase I trial. In the absence of dose-limiting toxicity, dose escalations were made at 3-week intervals at a fixed increment of 2 mg/m^2. A minimum of three patients were entered at each dose level. Doses were escalated for individual patients.

Study parameters.—Prior to each course of therapy every patient was assessed with a physical examination and a laboratory evaluation consisting of a cbc, SMA-12, electrolytes, prothrombin time, partial thromboplastin time, creatine phosphokinase, ECG, and chest x-ray. During each course of therapy, laboratory studies included twice weekly cbcs and weekly liver function tests, prothrombin time, BUN, creatinine, and creatine phosphokinase. Patients who received multiple courses of DAD had an echocardiogram repeated after every third course of therapy. Patients were considered evaluable for toxicity if they received a minimum of one dose of DAD and were followed for at least 3 weeks after treatment. Standard response criteria were used to evaluate antitumor effect when measurable disease was present.

Study termination.—Individual patients were removed from study when toxic effects were unacceptable or objective tumor progression occurred. This phase I study was terminated when the maximal tolerated dose was established.

RESULTS

Among 39 patients entered into this study, there were two with early deaths, two with protocol violations, and six with acute leukemia who were not evaluable for hematologic toxic effects (table 1). For the 29 patients evaluable for toxicity, the median age was 45 years (range, 1.5–77). Most patients were ambulatory, and all had received prior therapy. Seven patients had adenocarcinoma of the breast, five had malignant melanoma, three had soft tissue sarcoma, and two each had squamous cell carcinoma of the head and neck, adenocarcinoma of the colon, and neuroblastoma. Other diagnoses included adenocarcinoma of the ovary, pancreas, lung, and stomach; small cell carcinoma of unknown primary; thymoma; clear cell carcinoma of the vagina; and hepatoblastoma (table 2).

TABLE 1.—Patient characteristics

	No. of patients
Entered	39
Evaluable for toxicity	29
Evaluable for response	14
Inevaluable	10
Early death	2
Protocol violation	2
Leukemia	6
Age in yrs	
Median: 45	
Range: 1½–77	
Performance status*	
0–2	23
3–4	6
Prior therapy	
Chemotherapy only	13
Radiation therapy only	1
Both	15

*Eastern Cooperative Oncology Group criteria. 0–2 = ambulatory; 3–4 = nonambulatory.

TABLE 2.—Solid tumors

Tumor	No. of patients
Breast	7
Melanoma	5
Soft tissue sarcoma	3
Head/neck	2
Colon	2
Neuroblastoma	2
Miscellaneous	8

Toxicity

The dose-limiting toxic effect was leukopenia (table 3), which was both dose-related and reversible. The median nadir Day for leukopenia was Day 12, with full recovery always occurring by Day 21. There were no

TABLE 3.—Toxicity: leukopenia

Dose (mg/m^2/24 hrs)	No. of patients	No. of courses	No. of courses with wbc count nadir (x 10^3 cells/mm^3) of —				Nadir Day (range)
			≥ 3	2–2.9	1–1.9	< 1.0	
4	3	3	3	—	—	—	—
6	3	3	3	—	—	—	—
8	6	10	5	3	2	—	12 (11–15)
10	6	6	5	1	—	—	11
12	24	39	16	9	11	3	12 (7–17)
13	2	2	1	1	—	—	13
14	2	2	—	2	—	—	12
15	1	1	—	—	1	—	11

infectious complications or fever requiring hospitalization; no patient required antibiotic therapy. Thrombocytopenia was less commonly observed than leukopenia and did not occur at doses of ≤ 10 mg/m^2. Five of 44 courses at ≥ 12 mg/m^2 resulted in platelet counts of < 100,000/mm^3. The platelet count nadir occurred on Days 8–16, with full recovery by Day 21; there were no bleeding complications, and no patient required platelet transfusions. Three patients no experiencing dose-limiting myelosuppression at 12 mg/m^2 had dose escalations to higher levels. At 13 mg/m^2, one of two patients treated experienced thrombocytopenia with a platelet count nadir of 62,000/mm^3 on Day 8. The second patient treated at this dose level had no myelosuppression, and the dose was escalated to 14 mg/m^2. Two patients were treated at the 14-mg/m^2 dose level, and dose-limiting myelosuppression was not observed in either patient. One of these two patients subsequently received 15 mg/m^2 and developed leukopenia, with a wbc count nadir of 1600/mm^3 on Day 11.

Nonmyelosuppressive toxic effects consisted of mild phlebitis and blue discoloration of the vein in which the drug was infused. These effects occurred in four of 66 courses at various dose levels. There were four documented episodes of drug extravasation which produced no local toxic effects. No nausea, vomiting, diarrhea, mucositis, alopecia, or skin discoloration was observed. There were no elevations in hepatic enzymes, and no cardiac toxicity was noted on clinical examination, ECG, or serial echocardiograms.

Response

Fourteen of the 29 nonleukemic patients had objectively measurable disease and received at least two cycles of DAD at a dose of ≥ 12 mg/m^2. Partial responses were seen in two patients, including one who had adenocarcinoma of the breast with skin and lymph node metastases and another who had clear cell carcinoma of the vagina with malignant ascites and supraclavicular nodal metastases. These responses were of 3 and 2 months' duration, respectively.

Activity in leukemia.—Six patients with acute leukemia were treated with DAD at a dose of 12 mg/m^2 (table 4). Two of three patients with acute nonlymphocytic leukemia had a significant reduction in peripheral blast counts 3–12 days after treatment. One of two patients with acute lymphocytic leukemia had a significant reduction in peripheral blast count 14 days after therapy, without associated reduction in bone marrow blast count or cellularity. In one patient with mycosis fungoides in Sézary's leukemic phase, the initial blast count fell 16 days after therapy.

TABLE 4.—Leukemias

Disease*	Initial peripheral count		Peripheral count after DAD		
	Cells × 10^3	% blasts	Cells × 10^3	% blasts	Day
ANLL	69	88	19	84	3
ANLL	22	88	0.350	24	12
ANLL	1	5	1	5	—
ALL	260	90	0.500	20	14
ALL	0.900	36	1.3	5	8
MF/Sézary	145	75	64	89	16

*ANLL = acute nonlymphocytic leukemia; ALL = acute lymphoblastic leukemia; and MF/Sézary = mycosis fungoides with Sézary cell leukemia.

DISCUSSION

In a recently completed phase I trial of DAD given by rapid iv infusion repeated every 28 days, dose-limiting leukopenia was noted at 12 mg/m^2 (4). Other toxic effects observed were mild and included phlebitis with blue discoloration of veins, nausea, vomiting, and transient elevations of SGOT. A single partial response was noted in a patient with adenocarcinoma of the lung.

The results of our phase I trial of DAD given as a 24-hour continuous infusion every 21 days are similar to those recently reported. We, too, noted dose-limiting leukopenia at 12 mg/m^2; thrombocytopenia was less frequent and less pronounced in our study, and we did not observe a transient elevation of SGOT. However, the additional expense and patient discomfort produced by the longer drug infusion do not seem to be justified by the minor differences in toxicity observed. We would therefore recommend that phase II trials of DAD employ a short drug administration time rather than a 24-hour continuous infusion. It should be emphasized that this recommendation is based entirely on comparable toxic effects; however, the therapeutic efficacy of the 24-hour infusion method of drug delivery has not been evaluated.

The reduction in peripheral blast counts noted in four of the six patients with acute leukemia warrants further investigation. Since the only significant toxic effect noted to date is myelosuppression, we plan to explore the use of higher dose levels in a population of patients with refractory leukemias.

DAD has an electron-rich planar ring structure and has been demonstrated to be an intercalator of DNA. These are features which are common to the anthracycline antibiotics. This similarity naturally raises the question of the potential of DAD to produce cardiac toxicity. To date, published animal toxicology studies have not demonstrated cardiac toxicity, and no adverse cardiac effects have been noted clinically (4). The nature of a phase I study precludes the administration of large cumulative doses to a significant number of patients; therefore, the question of cardiac toxicity remains unanswered. It should be noted that 25 of the 39 patients in this study had received extensive prior anthracycline therapy (median dose, 400 mg/m^2; range, 120–480). All of these patients had normal parameters of cardiac function at the time of entry in our study, and none showed clinical deterioration of cardiac function related to the administration of DAD.

The largest cumulative DAD dose administered to a single patient at our institution to date is 102 mg/m^2. This patient had received a total cumulative doxorubicin dose of 450 mg/m^2 prior to entry into this study. He has no clinical evidence of congestive heart failure, and his echocardiogram is normal.

The maximally tolerated dose of DAD given as a 24-hour continuous infusion is 12 mg/m^2 every 21 days. We recommend that phase II trials be initiated at this dose level.

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Combination Chemotherapy With Amsacrine (AMSA) and Cyclocytidine in Refractory Childhood Leukemia: Preliminary Observations of a Phase II Study[1,2]

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The combination of daunorubicin and cyclocytidine for reinduction in childhood acute nonlymphoblastic leukemia (ANLL) in relapse has a remission rate of 75% M1 and M2A marrows; however, the duration of this remission is short (1). In an attempt to continue this high reinduction rate and to improve the duration of remission in ANLL, a phase II trial of amsacrine (AMSA), a new agent, and cyclocytidine for induction and AMSA and etoposide for maintenance was conducted by the Department of Pediatrics at the Memorial Sloan-Kettering Cancer Center. The response of patients with acute lymphoblastic leukemia (ALL) in relapse to AMSA alone and to cytarabine indicated that the combination of AMSA-cyclocytidine might be effective in ALL; therefore, this study was also open to these patients.

AMSA is an acridine derivative which has antitumor, immunosuppressive, and antiviral properties (2–4). The response rate in previously treated patients with acute leukemia was approximately 20% when AMSA was used as a single agent (5–7). Mucositis, nausea and vomiting, phlebitis, myelosuppression, hepatic dysfunction, and sepsis associated with periods of neutropenia were the major toxic effects. Echocardiogram changes occurred and some patients developed congestive heart failure, often in conjunction with sepsis or after receiving other cardiotoxic agents (8,9).

Cyclocytidine is an anhydro derivative of cytarabine and is resistant to deamination by pyrimidine nucleoside deaminase (10). The remission rate with cyclocytidine alone in relapsed patients was not as good as with cytarabine alone, but in combination with daunorubicin results were comparable (11,12). Unlike cytarabine, cyclocytidine can be given as an sc injection once a day and does not require continuous infusion to maintain cytotoxic levels of drug in the plasma. Jaw pain, nausea and vomiting, hepatic dysfunction, mucositis, local reaction at the injection site, and hypotension were the major toxic effects (10,12).

Etoposide is an epipodophyllotoxin which is active in children with acute leukemia in relapse (13,14). Toxic effects consist of nausea and vomiting, diarrhea, mucositis, hypotension, and local reaction at the injection site. Doses > 100 mg/m^2/day did not improve the remission rate but did increase the toxicity (15). Therefore, a dose of 100 mg/m^2/day was chosen for use in this study.

The objectives of this phase II combination protocol were (*a*) to determine the reinduction rate with AMSA-cyclocytidine; (*b*) to determine the duration of remission with AMSA-etoposide; and (*c*) to compare, retrospectively, the toxicity of these combinations with other agents, particularly in patients who had received previous anthracycline therapy.

MATERIALS AND METHODS

Sixteen pediatric patients (seven with ALL, six with ANLL, and three with juvenile chronic myeloid leukemia [JCML] in blastic crisis) were entered in the induction phase of the protocol. There were 12 males and four females. Patients ranged in age from 2 to 14 years (median, 7). All patients had previously received cytarabine and anthracycline (from 112 to 540 mg/m^2 of doxorubicin or its equivalent) therapy. All patients had an M3 bone marrow rating (*see* below) at the time of entry in the study and most had leukemic blasts in the

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peripheral blood. Eight patients had had from three to five previous bone marrow relapses, and two of these patients had a history of testicular relapse. One patient had been in prolonged bone marrow relapse and had a concomitant testicular relapse at the time of entry in this study. Two patients were in first relapse and two had had no response to previous induction chemotherapy protocols. Two patients were in bone marrow relapse after initial treatment for the blastic phase of JCML. One patient had associated central nervous system disease at the time of entry in the study.

Baseline studies included bone marrow and peripheral blood examination, echocardiogram, chest x-ray, and liver function tests. Bone marrow aspiration and biopsy were repeated 14 days after Day 1 of each treatment course. Echocardiograms were repeated before every other course of treatment or more frequently if indicated clinically. Liver function studies including LDH, bilirubin, alkaline phosphatase, 5′ nucleotidase, SGOT, and γGPT were performed at least once a week.

The induction phase of the protocol consisted of AMSA at a dose of 75 mg/m^2 iv over 1–2 hours on Days 1–5 and cyclocytidine at a dose of 600 mg/m^2 by sc injection on Days 1–7. Courses were repeated every 14–21 days, depending on the cellularity of the bone marrow and the percentage of leukemic blasts. If the bone marrow on Day 14 was moderately or markedly hypocellular with > 15% blasts, 3 days of AMSA and 5 days of cyclocytidine were given; if the bone marrow was slightly hypocellular, normocellular, or hypercellular with > 15% blasts, a full 7-day course was given as in Course 1. If the bone marrow showed < 15% blasts, the next course was given if the bone marrow was slightly hypocellular or normocellular, but was delayed, usually for 1 week, if the bone marrow was markedly hypocellular. Patients could receive up to four courses of induction therapy, depending on response to therapy.

The maintenance protocol consisted of AMSA at a dose of 100 mg/m^2 iv over 1–2 hours on Day 1 and etoposide at a dose of 100 mg/m^2 iv over 1 hour on Days 1–5. Maintenance courses were repeated every 21 days.

Patients were considered evaluable for response if they had completed two courses of treatment. All 34 induction courses were evaluated for toxicity. Toxicity was graded according to a schedule modified from Miller et al (16). A course was considered not evaluable for certain toxic effects if the patient had evidence of a toxic effect (ie, mucositis, infection, hemorrhage, or cardiac) prior to entry in the study and that effect did not become more severe during the study.

A complete remission was defined as an M1 marrow, normal peripheral blood cell counts and physical examination, and no symptoms of disease. An M1 bone marrow was defined as < 5% blasts with normal cellularity. A bone marrow with < 5% blasts for ALL or < 15% blasts for ANLL but hypocellular was considered a partial response. For ALL, an M2 bone marrow was defined as 6%–25% blasts and an M3 marrow as > 25% blasts. For ANLL, an M2A bone marrow was defined as 6%–15% blasts, M2B marrow as 16%–39% blasts, and M3 marrow as > 39% blasts. Cellularity of the bone marrow was graded as markedly hypocellular, slightly or moderately hypocellular, normocellular, and hypercellular. Treatment decisions were based on bone marrow cellularity and blast percentage.

Hospitalization was not required for the purpose of receiving chemotherapy. Informed consent was obtained for each patient prior to entry in the study.

RESULTS

Among the 16 patients, there were two early deaths: one of recurrent *Pseudomonas* sepsis and one of central nervous system disease. One patient is too early in the course of treatment to evaluate. Thirteen patients were evaluable for response. Every patient who had achieved a hypocellular marrow on biopsy after the first course of treatment had a response. Of the eight patients who responded, two had three bone marrow relapses, two had four bone marrow relapses (one with testicular cancer also relapsed), one had five bone marrow relapses, two had no response to previous attempts at induction therapy, and one had JCML in blastic crisis in second relapse before entry in the study.

Table 1 shows the pattern of response. Of the evaluable patients with ANLL, one patient remained in

TABLE 1.—Results

Diagnosis	No. of patients		No. of responses*
	Total	Evaluable	
ANLL	6	6	2 CR (9,3+) 1 PR ($1^3/_4$)
JCML in blast crisis	3	2	1 CR ($2^1/_2$) 1 PR (1+)
ALL	7	5	3 CR (1,1,1)

*CR = complete response; PR = partial response.

remission for 9 months after three courses of induction therapy. One patient with JCML in blastic crisis received three courses of induction therapy with progressive improvement and achieved a complete remission. He received three cycles of maintenance therapy and remained in remission for $2^1/_2$ months. Patient 3 achieved remission after two courses of induction therapy and continues in complete remission on maintenance therapy for > 3 months. Patient 4 had a partial remission with a hypocellular marrow but < 5% blasts in the bone marrow and, for the first time, absence of her aneuploid cell line as determined by flow cytometry while receiving AMSA-cyclocytidine. Her bone marrow was hypocellular from the time of diagnosis of refractory anemia with excess blasts 2 years earlier. At the time of her death from presumed viral infection, her bone marrow was without evidence of leukemia. One patient with JCML in blastic crisis who is still in the induction phase has had a good response thus far, with a decrease in blast percentage to 14%. Of the three patients who did not achieve a remission, one had a hypocellular marrow with a decrease in blast percentage from 77% to 16% after Course 1, but Course 2 was delayed and he did not respond further. Two patients had hypercellular marrow after Course 1 and did not respond to Course 2.

Three of five evaluable patients with ALL achieved complete remissions after two courses of induction therapy and each patient remained in remission for 1 month. For two of these patients AMSA-etoposide maintenance therapy was not begun, and one patient had a delay in beginning maintenance therapy and an interruption in treatment because of varicella zoster exposure. One patient is in the induction phase and is too early to evaluate. Two patients had hypercellular marrows after Course 1 and did not respond to Course 2.

Table 2 shows the side effects for 34 evaluable induction courses. Mucositis was minimal, occurred in only six courses, and was not severe enough to interfere with oral intake. Jaw pain responsive to Benadryl, Vistaril, or codeine occurred in 22 courses. Local reactions at the injection site were not observed. Nausea and vomiting was not severe. Hepatic dysfunction was, for the most part, mild or transient.

Infections have consisted of fever associated with periods of neutropenia in about one-half of the courses (Grade 2 toxicity), with two cases of pneumonia and five cases of documented bacterial sepsis. Five patients received granulocyte transfusions. There was one case of sinusitis and one of inguinal adenitis. One-half of all serious infections occurred in the four patients who had no response to therapy.

Hemorrhage was infrequent and occurred in two patients as epistaxis and in one as gastrointestinal bleeding in each of his two courses. No patient died of hemorrhage.

All patients had cardiac evaluations with echocardiograms done before and after treatment with AMSA. Cardiac changes consisted of a decrease in fractional shortening on echocardiogram to 26%–28% (normal, ≥ 29%) in three patients with previous anthracycline doses of 530, 210, and 150 mg/m^2. Changes were reversible when AMSA was discontinued or sepsis improved. One patient had a decrease in fractional shortening on the day of her death from sepsis, but her prior echocardiograms were normal after two courses of AMSA-cyclocytidine. She had also received a course of AMSA alone earlier in her treatment without any echocardiographic changes. One patient with a previous anthracycline dose of 540 mg/m^2 and a baseline echocardiogram that was borderline developed changes on echocardiogram and congestive heart failure during

TABLE 2.—Toxicity in 34 induction treatment courses*

	Not evaluable	Grade—				
		0	1	2	3	4
Mucositis	1	27		6		
Jaw pain		12	17	5		
Nausea and vomiting		14		20		
Hepatic						
Bilirubin, alkaline phosphatase		31	1	1		1
SGOT, γGPT, 5'nucleotidase		21	5	4	3	1
Infection	5	4		14	7	4
Hemorrhage	1	29		2	1	1
Cardiac	1	29	3		1	

*Values = No. of courses.

gram-negative sepsis and shock. Cardiac failure and echocardiogram changes improved with improvement in clinical condition; the patient received further therapy with AMSA-cyclocytidine and remained without cardiac problems.

DISCUSSION

Previous studies with AMSA as a single agent have shown it to be active in inducing remission in patients with acute leukemia in relapse (5–8,17–19). In phase I studies at the Memorial Sloan-Kettering Cancer Center, three of 13 patients with leukemia had complete or partial remission (8). At the M. D. Anderson Hospital and Tumor Institute, Houston, TX, 11 of 40 adult patients had complete or partial responses. In phase II studies in children at the Memorial Sloan-Kettering Cancer Center, five of 30 patients had complete or partial remissions (9). Responses obtained in these earlier studies with AMSA as a single agent often were achieved with doses higher than the 75 mg/m^2/day used in the current protocol. Toxicity was also greater. We sought to combine AMSA at a lower dose with other agents known to have activity in order to reduce toxicity and to improve the induction rate over that obtained with AMSA alone.

Cyclocytidine was chosen for induction because, in combination with other agents, it produced responses in acute leukemia in relapse (11). The remission rate in previous Children's Cancer Study Group retrieval protocols for ANLL using daunorubicin and cyclocytidine was impressive, although of short duration. With current front-line protocols using daunorubicin, relapsed patients may be refractory to this drug or have reached a cumulative dose prohibiting further therapy with the anthracyclines. Considering the design of the previous reinduction protocol, the present study replaced daunorubicin with AMSA and combined it with cyclocytidine. The mechanisms of action and toxic effects of AMSA may be different from those of the anthracyclines.

Etoposide as a single agent or in combination has also produced response in acute leukemia in relapse (13,14); its role in maintenance therapy in this study is undetermined and will require a larger number of patients entered in the maintenance phase of the protocol. Although this report focuses on the induction response to AMSA-cyclocytidine, a secondary but important question involves the role of maintenance therapy, if any, in the treatment of ANLL in relapse. One of our patients remained in remission for 9 months after receiving only induction therapy.

In this pilot study of 16 patients, we have shown that the combination of AMSA-cyclocytidine appears to be active and effective. It is often difficult to achieve a response in ANLL in relapse or in patients with ALL who have had multiple relapses. In this study, responses were seen in six of eight patients with ANLL and in three of five patients with ALL. Two of the patients who had a response had a diagnosis of JCML in blastic crisis, a group of patients particularly difficult to treat and generally refractory to most available chemotherapeutic agents. Of the patients who achieved remission, all except one had had at least three prior bone marrow relapses or no response to any previous chemotherapy.

Marrow cellularity on biopsy after one course may be predictive of ultimate response. Those patients who responded achieved hypoplasia on marrow biopsy. This is similar to the experience with AMSA alone, in which it was necessary to produce hypoplasia of the marrow to achieve response (12).

Side effects observed during induction courses were similar to those noted in earlier studies with AMSA or cyclocytidine alone; however, reactions or degree of toxicity was not as severe. In contrast to the experience with AMSA as a single agent (5,8,9), mucositis was minimal and did not cause a delay in treatment. Jaw pain and nausea and vomiting were well-controlled with medications. In previous reports on AMSA, hyperbilirubinemia was a frequent sign of hepatic dysfunction (5,7,17). One study related the elevation of bilirubin to episodes of sepsis. In this study, elevated bilirubin occurred in only one patient with presumed viral hepatitis who had episodes of marked hepatic dysfunction throughout the course of her disease. Cases of documented sepsis were not associated with hyperbilirubinemia. Echocardiographic changes were seen in four patients but, for the most part, were reversible with discontinuation of or a delay in treatment or with improvement in clinical condition. Further cardiac studies and longer follow-up will be needed to determine whether the cardiac changes seen with AMSA are more readily reversible than the changes seen with anthracyclines. It is not possible to conclude that the cardiac changes seen with AMSA are due to AMSA itself because in almost every instance echocardiographic changes and clinical problems occurred in patients who had received either substantial prior therapy with anthracyclines or who had concomitant sepsis. Infections were associated with periods of neutropenia and were treated with broad-spectrum antibiotics and with amphotericin B. Overall, most toxicity has been mild; moderate or severe toxicity, for the most part, has been transient or reversible.

We conclude that the combination of AMSA-cyclocytidine is effective in inducing remission in acute leukemia in relapse and that it may be most useful in ANLL. Preliminary results in patients with JCML are encouraging and warrant further evaluation. It is unlikely that the protocol will have a higher reinduction rate than daunorubicin-cyclocytidine in ANLL, but our present high response rate in this limited number of heavily pretreated patients is very encouraging. This protocol

may also be useful for relapsed patients who are transplant candidates in whom induction of complete remission is possible without cumulative toxicity. To enroll a sufficient number of patients to evaluate fully the remission rate, toxicity, and time to first event for these drug combinations, this protocol has been opened for group-wide study in ANLL by the Children's Cancer Study Group.

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Disposition of 9-β-D-Arabinofuranosyl-2-fluoroadenine 5′-phosphate in Mice and Dogs[1,2]

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The metabolic disposition of 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-phosphate (2-F-araAMP) has been studied in mice and dogs after iv administration. Following injection of 40 mg/m² into mice, serum levels of 2-F-araAMP fell, with apparent half-lives of 0.7 mins for the α phase and 21 mins for the β phase. Rapid dephosphorylation of the compound resulted in high levels of the nucleoside, 9-β-D-arabinofuranosyl-2-fluoroadenine (2-F-araA), which disappeared from serum in two phases, with half-lives of 31 and 114 mins. 9-β-D-Arabinofuranosyl-2-fluorohypoxanthine (2-F-araHx) was also present in serum. After administration of 500 mg/m² of 2-F-araAMP to mice, the apparent half-lives for the parent compound were 2.5 and 27 mins. Serum levels of 2-F-araA fell, with apparent half-lives of 36 and 185 mins. Tissue distribution studies revealed that, in mice administered 40 mg/m², the liver, kidney, and spleen contained the highest levels of 2-F-araAMP. For most tissues, elimination of 2-F-araAMP occurred exponentially but at a slower rate than in serum. As in serum, the major tissue metabolite was 2-F-araA; other metabolites identified were 2-F-araHx, 2-fluoroadenine (2-F-A), and polyphosphorylated derivatives of 2-F-araA. In mice dosed with 500 mg/m², the tissue distribution of metabolites was similar, but the levels were about tenfold higher. With both doses, 2-F-araAMP, 2-F-araA, and 2-F-araHx were recovered in urine. In dogs administered 40 mg/m², serum levels of 2-F-araAMP fell rapidly in an initial phase of 5 mins and in a second phase of 30 mins. As in mice, the major serum metabolite was 2-F-araA, which disappeared in two phases of 16 and 97 mins. After administration of 500 mg/m² to dogs, the apparent half-lives for 2-F-araAMP in serum were 9 and 51 mins. For 2-F-araA, a single phase of disappearance of 89 mins was evident. In dog serum, as compared with mouse serum, a greater percentage of the administered compound was metabolized to 2-F-araHx. During 24 hrs following administration of either 40 or 500 mg/m² of 2-F-araAMP to dogs, < 2% of the dose was excreted in urine as unchanged drug. Approximately equal percentages of 2-F-araA and 2-F-araHx were recovered in urine after both doses. The levels of 2-F-A in urine comprised about 2% of the radioactivity after administration of either the low or the high dose. The studies demonstrate that 2-F-araAMP undergoes rapid dephosphorylation in both mice and dogs. Deamination of the dephosphorylated product is more rapid in dogs than in mice. [Cancer Treat Rep 67:445–456, 1983]

The arabinosyl nucleoside, 9-β-D-arabinofuranosyladenine (araA), is a potent antiviral agent, the cytotoxicity of which correlates with its phosphorylation to the 5′-triphosphate (1–3). The biologic activity of araA, however, is limited, due to rapid deamination by adenosine deaminase (4–6). Deamination gives rise to 9-β-D-arabinofuranosylhypoxanthine (araHx), a compound that is less cytotoxic than araA (7). Following the observation that 2-fluoroadenosine was not a substrate for adenosine deaminase from calf intestinal mucosa (8), the

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[4]We appreciate the assistance of Dr. Robert Struck in the purification of the radiolabeled compound, Dr. Harris Lloyd in the calculation of the pharmacokinetic parameters, and Mr. Thomas Herren in the assay of radioactive samples.
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synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine (2-F-araA) was accomplished (9). 2-F-araA is resistant to deamination by adenosine deaminase from both P388 and L1210 leukemia cells (10) and is an effective agent against L1210 (10) and human lymphoblastic (11,12) leukemias. Similar to araA, the inhibition of DNA synthesis produced by 2-F-araA correlates with the accumulation of 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-triphosphate in leukemic lymphoblasts (11). In vivo, 2-F-araA gives rise to phosphorylated derivatives, to 2-F-arabinofuranosylhypoxanthine (2-F-araHx), and to the toxic derivative, 2-fluoroadenine (2-F-A) (13,14). The limited solubility of 2-F-araA led to the synthesis of its 5′-phosphorylated derivative, 2-F-araAMP, for which activity against animal tumors has been established (15). It currently is scheduled to undergo phase I clinical trials.

In view of the observations that araAMP enters cells without dephosphorylation (16) and is more resistant to deamination than the nucleoside, araA (17), it is of interest to determine the disposition of 2-F-araAMP in animals. The present study describes the comparative disposition of this compound after iv injection of 40 and 500 mg/m^2 into mice and dogs.

MATERIALS AND METHODS

2-F-araAMP was synthesized by Dr. J. A. Montgomery of the Southern Research Institute (Birmingham, AL) and tritiated by New England Nuclear Corp (Boston, MA). Further purification of the labeled product was accomplished in our laboratories by Dr. R. F. Struck. The radiochemical purity of the [^{3}H]2-F-araAMP was determined by high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC).

For HPLC, a Waters Associates (Milford, MA) high-pressure liquid chromatograph equipped with two Model 6000 pumps, a Model 660 gradient programmer, and a Partisil ODS-2 column (Whatman Inc, Clifton, NJ) was used. Resolution of 2-F-araAMP and its metabolites was achieved at room temperature with a gradient elution (Waters Program 2; 5% Solvent B, initial, to 95% Solvent B, final) using 5 mM $(NH_4)H_2PO_4$, pH 2.8 (Solvent A) and methyl alcohol:water (1:3) (Solvent B) at a flow rate of 1 ml/min. One-milliliter fractions of the eluting material were collected into scintillation vials and, after the addition of 10 ml of ScintiVerse I (Fisher Chemical Co, Fair Lawn, NJ), were counted in a liquid scintillation spectrometer equipped with an external standard. Eluting materials also were detected by UV absorption at 254 nm. For this system, in aqueous solution, the retention times for authentic standards of 2-F-araAMP, 2-F-araHx, 2-F-araA, and 2-F-A were 11, 15, 24, and 27 mins, respectively; the coefficient of variation of the individual retention times was < 5%. Similar elution patterns and resolution were obtained following injection of standard solutions prepared in urine or tungstate deproteinizing reagent (TDR) (18). By UV absorption, the lower limit of detectability of unlabeled 2-F-araAMP and related compounds was of the order of 5–10 ng.

For TLC, ITLC-SA sheets (Gelman Instrument Co, Ann Arbor, MI) were developed in the following solvent systems: isopropyl alcohol/ammonium hydroxide/water, 7:1:2 (vol/vol/vol) (System A); and chloroform/methyl alcohol/water, 50:50:1.5 (vol/vol/vol) (System B). Unlabeled 2-F-araAMP, detected by UV light at 254 nm, was used as the reference compound. The solvent front was allowed to migrate 15 cm. After drying, the sheets were cut into 0.6-cm strips, starting 0.9 cm behind the origin and ending about 1 cm beyond the solvent front. The strips were placed in scintillation vials, to which were added 1 ml of 10% ethyl alcohol and, after about 30 mins, 10 ml of ScintiVerse I. The radioactivity content of each vial was determined by liquid scintillation spectrometry. 2-F-araAMP had R_f values of 0.57 and 0.15 in Systems A and B, respectively.

Animals used in this study were male BDF_1 mice weighing 18–25 g and female beagle dogs weighing 7.8–10.8 kg. All animals were denied food for 18 hrs prior to drug administration but were allowed water ad libitum. Equivalent doses of 40 or 500 mg/m^2 were injected iv into the tail vein of mice and into the saphenous vein of dogs. For the mouse studies, solutions of 2-F-araAMP (1.35 mg/ml for the low dose; 15 mg/ml for the high dose) were prepared in water to give injection solutions with specific activities of 66 μCi/mg (low dose) and 5.9 μCi/mg (high dose). Radiochemical purity of the solutions was > 96%. For the dog studies, solutions were prepared similarly but with specific activities of 88 μCi/mg (low dose) and 10.5 μCi/mg (high dose). Radiochemical purity of these solutions was 94%.

At selected times after dosing, blood was collected from the axillary region of mice anesthetized with diethyl ether and from either a brachial or saphenous vein of dogs. The samples were immediately placed in an ice bath to clot and then centrifuged to allow collection of serum. A portion (0.01–0.05 ml) of serum was added to a scintillation vial and was counted in a liquid scintillation spectrometer after the addition of 15 ml of Liquifluor (New England Nuclear Corp) containing 7% Bio-Solv Formula BBS-3 solubilizer (Beckman Instruments, Palo Alto, CA). To another portion, four volumes of TDR were added, and the supernatant obtained after centrifugation was assayed by HPLC, as described above. All urine collections were made by catheter, and radioactivity determinations on the samples were made as described for serum, with the exception that ScintiVerse I was added as the scintillator. Urine samples were assayed, without treatment, by either HPLC or TLC for levels of parent compound and metabolites. For mice, various tissues and organs were

collected separately at selected times after dosing, blotted on paper towels, and immediately frozen on dry ice. After being weighed, the tissues were homogenized, without prior thawing, in four volumes of TDR using a Polytron homogenizer equipped with a P-10 generating head (Brinkmann Instruments, Westbury, NY). Portions (0.1 ml) of each homogenate were digested with 0.5 ml of Soluene 350 tissue solubilizer (Packard Instruments, Downers Grove, IL) and counted in a liquid scintillation spectrometer after the addition of 15 ml of Liquifluor. Supernatants obtained after centrifugation of the homogenates were assayed by HPLC.

Pharmacokinetic half-lives for 2-F-araAMP and metabolites were estimated using a modified form of NONLIN (19) and CSTRIP (20). The data were fitted to one-, two-, and three-compartment open models. A model was accepted as the best fit if an additional term, or compartment, failed to reduce significantly ($P < 0.05$) the weighted sums of squared errors as estimated by F test with appropriate degrees of freedom. Statistical weights were determined from the measured concentrations and were the same for each model.

RESULTS AND DISCUSSION

In in vitro experiments, 2-F-araAMP was stable when incubated for 24 hrs at 37°C in either saline, water, or phosphate buffer at neutral pH. Substantial (> 70%) dephosphorylation occurred in 1 N perchloric acid, mouse serum, or mouse urine at 37°C. In mouse urine, degradation did not occur when the incubation temperature was 4°C. With [^{3}H]2-F-araAMP, no detectable release of label in the form of ^3H_2O occurred under any of the above incubation conditions. The observed rapid in vitro dephosphorylation of 2-F-araAMP in serum necessitated immediate precipitation of serum samples obtained in vivo. In TDR, at neutral pH, the compound was stable for several months when stored at –20°C.

Following iv injection of 40 mg/m^2 (13.5 mg/kg) of [^{3}H]2-F-araAMP into mice, 2-F-araAMP disappeared

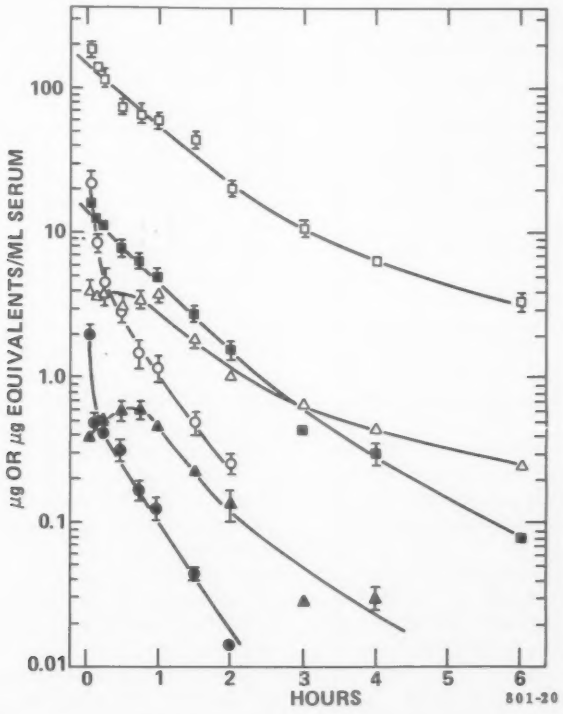

FIGURE 1.—Serum levels of 2-F-araAMP and major metabolites in mice after iv administration of either 40 mg/m^2 (closed symbols) or 500 mg/m^2 (open symbols). ○,● = unchanged 2-F-araAMP; □,■ = 2-F-araA; Δ,▲ = 2-F-araHx. Values on the vertical axis are μg/ml of serum for 2-F-araAMP and μg-equivalents/ml of serum for metabolites. Values represent mean ± SD for at least 3 mice at each time of assay. For some points, SD was too small to be represented.

Table 1: Pharmacokinetic Parameters Derived from Measurement of [^{3}H] 2-F-araAMP or [^{3}H] 2-F-araA in Serum of Mice and Dogs Administered [^{3}H] 2-F-araAMP[a]

Compound	A_o (μg/ml)	α (min^{-1})	B_o (μg/ml)	β (min^{-1})	$t_{1/2_\alpha}$ (min)	$t_{1/2_\beta}$ (min)	V_c[b] (ml)	V_B[c] (ml)	K_{10}[d] (min^{-1})	K_{12}[d] (min^{-1})	K_{21}[d] (min^{-1})	C x t[e] (μg min/ml)	Clearance[f] (ml/min)
MICE													
40 mg/m^2													
2-F-AraAMP	95.6	0.9433	0.87	0.0328	0.7	21.2	3.1	73.4	0.7718	0.1642	0.0400	124.8	2.40
2-F-AraA	15.5	0.0223	0.71	0.0061	31.1	113.9	18.5	60.6	0.0200	0.0016	0.0068	813.4	0.37
500 mg/m^2													
2-F-AraAMP	70.2	0.2813	5.2	0.0258	2.5	26.9	47.8	309.1	0.1668	0.0968	0.0435	451.9	7.97
2-F-AraA	147.5	0.0194	12.4	0.0037	35.7	184.9	22.5	88.0	0.0147	0.0035	0.0050	10912.0	0.33
DOGS													
40 mg/m^2													
2-F-AraAMP	0.21	0.1298	0.11	0.0228	5.3	30.5	66748.0	142960.0	0.0487	0.0432	0.0606	6.64	3254.0
2-F-AraA	1.6	0.0442	2.0	0.0072	15.7	96.6	5958.0	9552.7	0.0115	0.0123	0.0276	315.2	68.5
500 mg/m^2													
2-F-AraAMP	2.2	0.0758	0.89	0.0135	9.2	51.5	82113.0	196520.0	0.0322	0.0254	0.0317	94.5	2646.0
2-F-AraA*	10.4	0.1494	34.0	0.0077	4.6	90.3	5638.0	7243.5	0.0099	0.0309	0.1163	4496.8	55.6

[a] Values are mean values for at least three mice and three dogs at each dose level. Data were fitted to a biexponential equation of the form $C = A_o e^{-\alpha t} + B_o e^{-\beta t}$, where C is the concentration at any time, t. A_o and B_o are the zero time intercepts for the phases of elimination and α and β are the rate constants describing the decay of those phases of elimination.

[b] V_c = volume of distribution of the central compartment

[c] V_B = total body volume of distribution

[d] Values represent the microscopic rate constants for total body elimination (k_{10}), distribution from the central compartment to the peripheral compartment (K_{12}) and distribution from the peripheral to the central compartment (K_{21}).

[e] $C \times t = \int_o^\infty c\,dt$ [f] Clearance = dose/C x t *Two-compartment model was not a significantly better fit than a one-compartment model.

from serum in two phases, with apparent half-lives of 0.7 and 21 mins (fig 1). The rapid dephosphorylation of the compound resulted in high serum levels of 2-F-araA. Within 5 mins after dosing, peak serum levels of 2-F-araA of 15.9 μg-equivalents/ml were obtained; thereafter, levels of this metabolite fell with an initial disappearance phase of 31 mins and a second phase of 114 mins. The rate of disappearance of 2-F-araA after 2 hrs was equivalent to that previously observed following iv administration of an equivalent dose of [^{3}H]2-F-araA to mice (13). Other pharmacokinetic parameters describing the disposition of these two compounds are listed in table 1. HPLC analysis of collected serum samples also established the presence of a metabolite having a retention time similar to that of authentic 2-F-araHx. The levels of 2-F-araHx were relatively low, comprising, at peak levels, 8% of the radioactivity. This compound previously was identified in the urine of mice administered an equivalent dose of 2-F-araA (14). Other minor metabolites in serum, which were not identified, accounted for < 5% of the radioactivity present at any time. After administration of 500 mg/m^2 (165 mg/kg) of [^{3}H]2-F-araAMP to mice, serum levels of unchanged drug fell from 21.6 μg/ml at 5 mins, with an apparent half-life of 2.5 mins for the α phase and 27 mins for the β phase (fig 1). At this dose, the calculated clearance rate of 2-F-araAMP from serum was somewhat faster than after administration of the low dose. This difference in clearance rates was most probably due to an underestimation of clearance for the low dose. As shown in table 1, after administration of 40 mg/m^2, the value for A_o computed from the weighted corrected sum of squared observations is substantially greater than that predicted by theory. This apparent overestimation was probably a consequence of the extremely rapid dephosphorylation of 2-F-araAMP, which made accurate measurement of initial serum levels difficult. The slightly longer half-lives for 2-F-araAMP at this dose may indicate that saturation of the receptor sites on the serum phosphatases or 5′-nucleotidases had occurred. For 2-F-araA, the major serum metabolite, no saturable elimination processes were detectable, and levels of this metabolite fell in two phases, with apparent half-lives of 36 and 185 mins. 2-F-araHx was present at levels approximately tenfold higher than after the low dose. With both doses, 2-F-A, a metabolite previously identified in urine after 2-F-araA administration (13,14), was not detectable in serum. It should be noted that at a dose level of 500 mg/m^2, some mortality (about 10%) was observed following iv administration of 2-F-ara AMP. In most instances death occurred within several minutes after dosing. The etiology of this was not investigated but may have been attributable to the salts in the preparation since the dose was well below the LD10 determined for mice given a single ip dose (F. M. Schabel, Jr, unpublished results) and was equivalent to the dose of 2-F-araA given in a previous study, in which no mortality was observed (13).

With both administered doses, some exchange of ^{3}H-label occurred in mice at the late time periods. The presence of ^3H_2O in serum was indicated by the HPLC elution patterns and differences in the radioactivity content of samples counted both before and after drying. At 3 hrs after dosing, 45% of the small amount (< 2% of the dose) of radioactivity remaining in serum was present as ^3H_2O for mice dosed with 40 mg/m^2; the value was 20% for those dosed with 500 mg/m^2. These differences in the extent of ^{3}H-exchange may have been due to saturation, at the high dose, of the receptor sites interacting with the label. Since ^3H_2O was not detectable at periods up to 60 mins after dosing, the exchange of label should have had a minimal effect on the computation of half-lives for 2-F-araAMP.

The tissue distribution of 2-F-araAMP and its major metabolites in mice dosed with 40 mg/m^2 of [^{3}H]2-F-araAMP is shown in figure 2. Within 5 mins after dosing, 2-F-araAMP was detected (in concentrations ranging from 1.1 to 11.4 μg/g) in all tissues assayed. In the liver, 2-F-araAMP levels increased with time, suggesting that intracellular synthesis of the compound from the nucleoside was occurring. For the other tissues studied, the levels of 2-F-araAMP declined exponentially with time but at a slower rate than in serum. As in serum, the major metabolite in the tissues was 2-F-araA. This metabolite constituted > 60% of the radioactivity present in liver, kidney, spleen, and small intestine at 60 mins. In several tissues (spleen, lungs, and heart) the rate of disappearance of 2-F-araA paralleled that for serum, but in others (liver and small intestine) the tissue to plasma ratio increased with time, indicating intracellular synthesis. At least three other metabolites were in tissues in varying concentrations. 2-F-araHx was present predominantly in the small intestine, cecum (not shown), and spleen; the lowest levels were in the heart and liver. Compounds having retention times equivalent to di- and tri-phosphorylated derivatives of 2-F-araA were present in varying amounts in all tissues assayed. Treatment of selected tissue samples with snake venom phosphodiesterase (Sigma Chemical Co, St. Louis, MO) allowed confirmation of the suggested structure of these metabolites. Following a 30-min incubation with phosphodiesterase, chromatographic analysis of treated samples revealed the absence of both 2-F-araAMP and more polar compounds (the presumed polyphosphorylated derivatives of 2-F-araA) and proportional increases in the levels of 2-F-araA. Levels of the less polar metabolites were unaffected by phosphodiesterase treatment. The highest levels of di- and tri-phosphates were in the liver, further confirming the phosphorylating capabilities of this tissue. In contrast, almost no di- or tri-phosphorylated nucleotides were in the spleen. The formation of cellular

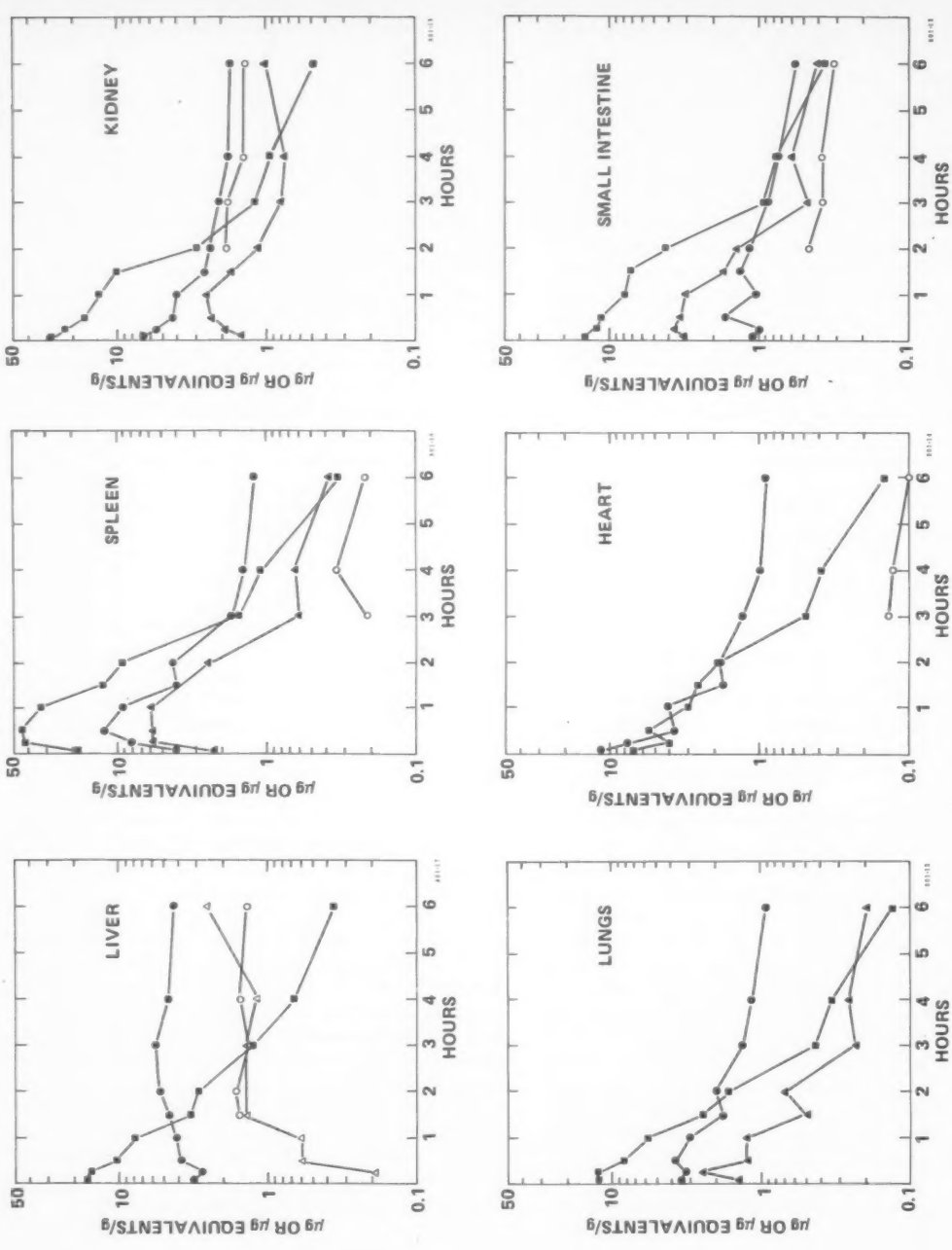

FIGURE 2.—Tissue levels of 2-F-araAMP and major metabolites in mice after iv administration of 40 mg/m². ● = 2-F-araAMP; ■ = 2-F-araA; ▲ = 2-F-araHx; ○ = 2-F-A; Δ = di- and tri-phosphorylated derivatives of 2-F-araAMP. Values on the vertical axis are μg/g for 2-F-araAMP and μg-equivalents/g for metabolites. Values are from pooled tissues of 3 mice at each time of assay. Metabolites not represented constituted < 5% of the radioactivity at a given time.

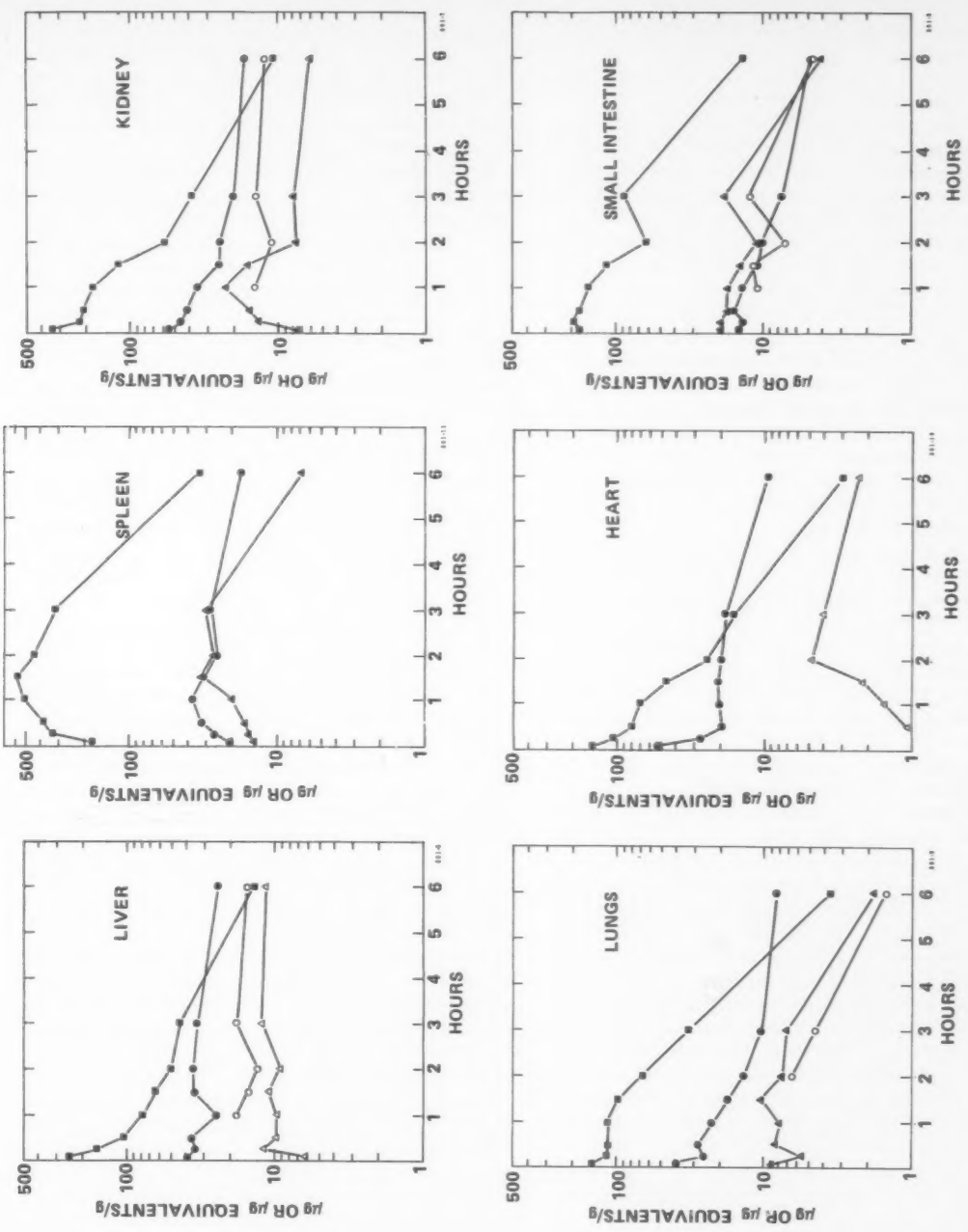

FIGURE 3.—Tissue levels of 2-F-araAMP and major metabolites in mice after iv administration of 500 mg/m^2. Symbols and values are same as for fig 2.

nucleotides following 2-F-araAMP administration would appear important in view of the fact that 2-F-araA has been shown to exert its cytotoxic effect after conversion to triphosphorylated nucleotides (11).

In mice dosed with 500 mg/m^2 of [^{3}H]2-F-araAMP (fig 3), the tissue distribution of radioactivity was similar to that after administration of 40 mg/m^2. For both doses, only small amounts of radioactivity appeared in brain, testes, and fat (data not shown). At the early times after drug administration, the levels of 2-F-araAMP and major metabolites (2-F-araA and 2-F-araHx) were, in general, about tenfold higher than after administration of 40 mg/m^2 (fig 3); however, with the high dose, tissues demonstrated a decreased rate of elimination of 2-F-araAMP and 2-F-araA. For the small intestine and spleen, there was, additionally, an increased half-life for 2-F-araHx. The apparent changes in tissue elimination rates coincided with slightly longer serum half-lives for these metabolites at the higher dose (fig 1) and indicated that, in tissues, either the elimination processes had become saturated or the rate of synthesis of the compounds was increased.

For both administered doses, a metabolite having an HPLC retention time similar to 2-F-A was found in small amounts, predominantly in liver and kidney, within 1 or 2 hrs after dosing and at later times. The highest amounts detectable at any time represented about 1% of the administered dose. This compound also has been identified in the urine of mice administered an equivalent dose of 2-F-araA (14). As discussed below, the formation of 2-F-A from 2-F-araAMP and its nucleoside may have important clinical implications in view of the reported toxicity of this compound.

Within 24 hrs, mice administered 40 mg/m^2 of [^{3}H] 2-F-araAMP excreted 1.1% of the dose in urine as unchanged drug, 89.4% as 2-F-araA, and 10.0% as 2-F-araHx (table 2). These results were consistent with the rapid serum dephosphorylation of 2-F-araAMP, resulting in the formation of 2-F-araA, and were reflective of the almost tenfold difference in serum levels of 2-F-araA and 2-F-araHx. In mice administered 500 mg/m^2, the pattern of metabolite excretion was similar to that for the low dose. Although present in tissue, 2-F-A was not detectable in mouse urine after either dose. This may have been related to the low solubility of the compound and to the observation that, in most mouse tissues, the levels of 2-F-A remained constant with time (figs 2 and 3).

It should be noted that initial results obtained for mice after administration of 500 mg/m^2 indicated that only about 70% of the dose had been excreted in the urine within 24 hrs. Dilution of the urine with water and warming to 65°C, however, allowed recovery of > 95% of the administered radioactivity. HPLC analyses demonstrated that the increased levels of radioactivity were attributable to increased percentages of 2-F-araA in the samples. These results indicated that 2-F-araA initially had precipitated in these samples. The limited solubility of 2-F-araA may have been responsible for the lower urinary recovery (< 80% of the dose) obtained in a previous study where 2-F-araA was the administered compound (14).

In dogs administered 40 mg/m^2 (2 mg/kg) of [^{3}H]2-F-araAMP, serum levels of 2-F-araAMP fell rapidly in an initial phase of 5 mins and in a second phase of 30 mins (fig 4). As in mice, the major metabolite in dog serum was 2-F-araA. This metabolite disappeared from serum in two phases, with apparent half-lives of 16 and 97 mins (table 1). Peak serum levels of 2-F-araA were comparable to those obtained after administration of an equivalent dose of 2-F-araA to dogs (13), and the second phase of disappearance was approximately equivalent in length to that previously reported for the β phase of this compound (13). Serum levels of 2-F-araHx increased during the first 60 mins after dosing and then fell with an apparent half-life of 113 mins. It is noteworthy that, in dogs as compared with mice, 2-F-araHx accounted for a greater percentage of the administered dose, an observation in agreement with data obtained following 2-F-araA administration (14). Other metabolites present in dog serum accounted for < 5% of the administered radioactivity. Following iv dosing of dogs with 500 mg/m^2 (25 mg/kg), the pattern of metabolite formation was similar to that seen after administration of the low dose, but serum metabolite levels were about tenfold higher (fig 5). The apparent half-lives for 2-F-araAMP were 9 and 43 mins. For 2-F-araA, elimination occurred in a single phase, with a half-life of 89 mins. Other pharmacokinetic parameters derived for the disposition of 2-F-araAMP and 2-F-araA in dogs are included in table 1. As indicated by the calculated values for clearance, the processes responsible for the elimination of either compound from serum were not saturated at the higher dose. For 2-F-araHx, as with the low dose, serum levels increased for 60 mins then fell, with an apparent half-life of 113 mins. Pharmacokinetic parameters for 2-F-araHx, derived for a monoexponential elimination phase, are included in table 3. No dose-dependent formation or elimination was observed for this metabolite. For both doses, no gross toxicity or mortality was observed in any treated dogs.

The data representing the 24-hr cumulative urinary excretion of 2-F-araAMP and metabolites in dogs administered either the low or high dose are shown in table 2. Following administration of 40 mg/m^2 of [^{3}H] 2-F-araAMP to dogs, < 2% of the dose was excreted into urine as unchanged drug, a percentage similar to that obtained for mice given an equivalent dose. Additionally, 41.5% of the dose was present as 2-F-araA and 44.2% as 2-F-araHx. The rates of elimination of these two metabolites into urine paralleled their rates of disappearance from serum (data not shown). In urine,

TABLE 2.—Urinary metabolites of 2-F-araAMP in mice and dogs administered [^{3}H]-2-F-araAMP iv*

	% dose administered			
	Mice		Dogs	
Compound†	40 mg/m^2	500 mg/m^2	40 mg/m^2	500 mg/m^2
2-F-araAMP	1.1 ± 0.3	7.2 ± 0.6	1.7 ± 0.4	1.6 ± 0.5
2-F-araHx	10.0 ± 0.8	6.6 ± 0.6	44.2 ± 4.8	43.7 ± 6.4
2-F-araA	89.4 ± 4.1	83.2 ± 0.2	41.5 ± 3.9	27.2 ± 9.8
2-F-A	ND‡	ND	1.6 ± 0.2	2.5 ± 0.7
Other	2.2 ± 0.2	6.2 ± 5.5	2.5 ± 0.6	2.1 ± 1.7
% dose recovered	103.3 ± 3.6	103.2 ± 5.3	91.6 ± 2.0	77.1 ± 18.2

*Data are for 5 mice dosed with 40 mg/m^2, 2 mice dosed with 500 mg/m^2, and 3 dogs dosed with either 40 or 500 mg/m^2. Mean ± SD is presented.

†Identification was by HPLC assay, as described.

‡ND = not detectable.

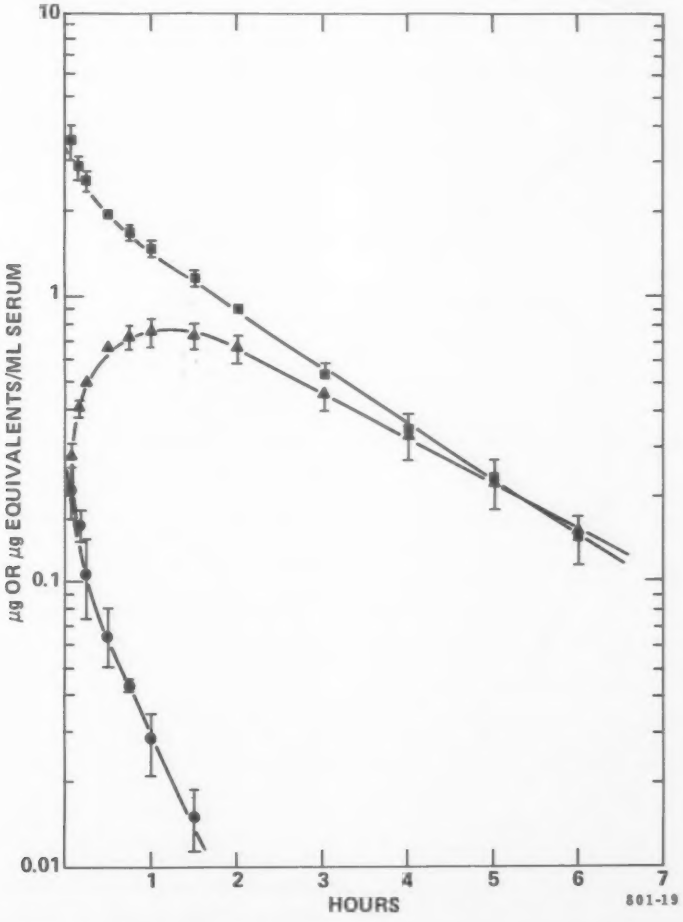

FIGURE 4.—Serum levels of 2-F-araAMP and major metabolites in dogs after iv administration of 40 mg/m^2. ● = 2-F-araAMP; ■ = 2-F-araA; ▲ = 2-F-araHx. Values on the vertical axis are μg/ml serum for 2-F-araAMP and μg-equivalents/ml serum for metabolites. Values represent mean ± SD for 3 dogs.

TABLE 3.—Pharmacokinetic parameters derived for 2-F-araHx in dogs administered [^{3}H]2-F-araAMP*

Dose (mg/m^2)	A_o ($\mu g/ml$)	K_e (min^{-1})	K_m (min^{-1})	$t_{1/2}$ (mins)	C × t† (μg min/ml)	Clearance† (ml/min)
40	1.4	0.0061	0.0304	113.5	187.0	115.5
500	17.9	0.0062	0.0272	112.5	2248.0	111.2

*Means values obtained from 3 dogs at each dose level. Data were fitted to a monoexponential equation of the form $C = A_o (e^{-K_e} - e^{K_m})$, where C is the concentration, A_o is the 0 time intercept, K_e is the elimination rate constant for 2-F-araA from 1 compartment, and K_m is the elimination rate constant for 2-F-araHx.

†For definitions, *see* footnotes [e] and [f] in table 1.

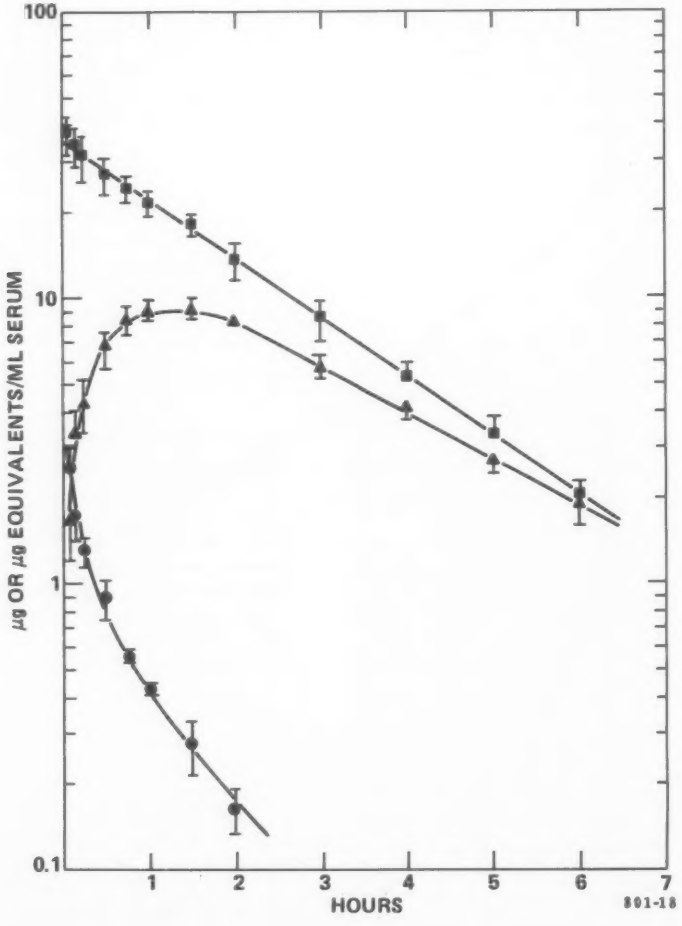

FIGURE 5.—Serum levels of 2-F-araAMP and major metabolites in dogs after iv administration of 500 mg/m^2. Symbols and values are same as for fig 4.

< 2% of the dose was 2-F-A. In dogs administered 500 mg/m^2, the percentages of 2-F-araAMP, 2-F-araA, and 2-F-araHx recovered in urine were 1.6, 27.2, and 43.7, respectively (table 2). Of the recovered radioactivity, the levels of 2-F-A comprised 2.5%, a value substantially lower than the value of 8.9% observed in dogs administered an equivalent dose of 2-F-araA (14). It should be noted that considerably more variation occurred in the rates of metabolite excretion when the high dose was administered to dogs, as compared with the low dose. The individual values for total recovery of administered radioactivity for the three dogs represented in table 2 were 64.6%, 68.7%, and 98.0%, respectively. The differences in recovery appeared to be related to the percentage of radioactivity excreted as 2-F-araA. For the dog with the highest recovery, 38.3% of the dose was 2-F-araA; the corresponding values for the other two dogs were 23.2% and 20.0%, respectively. These differences in elimination may be related to the substantially larger volumes of urine voided by the former dog, as compared with the latter two. As suggested by the results obtained with mice, 2-F-araA may precipitate in urine when large quantities are excreted in relatively small volumes of urine.

These studies demonstrate that in mice and dogs administered equivalent doses of 2-F-araAMP iv, differences exist in the extent of deamination and cleavage of 2-F-araAMP. In mice, few metabolites other than 2-F-araA are present in serum, indicating an apparent high affinity of serum phosphatases and/or 5′-nucleotidase for the parent compound. Other metabolites found in tissues include 2-F-araHx, 2-F-A, and polyphosphorylated nucleosides. In dogs, 2-F-araA is also the predominant metabolite, but relatively greater amounts of 2-F-araHx are found in serum and excreted in urine, indicating a greater capacity of the dog to deaminate the nucleoside. For both species, at the doses administered, the elimination of 2-F-araAMP and metabolites was dose-independent. The observed species differences in metabolism, although minor, might be expected to result in a differential cytotoxicity of 2-F-araAMP in various species since the metabolites produced exert pharmacologic activities distinct from each other and the parent compound.

The antitumor activity of 2-F-araHX has been evaluated in mice bearing L1210 leukemia cells (14). The compound possesses no antitumor activity and produces no gross toxicity. The deaminated product of araA, araHx, is also less cytotoxic than the parent compound (7). As such, in species in which a large percentage of an administered dose of 2-F-araAMP is metabolized to 2-F-araHx, one would predict that the minimum therapeutic (toxic) dose would be greater than in a species producing a small percentage of this metabolite. It is not yet known to what extent humans deaminate 2-F-araA or 2-F-araAMP. In a study where the 5′-phosphate of araA was administered to four patients, 35%–45% of the dose was recovered in the urine as araHx (21), results which indicate that humans can deaminate araA to the same extent that dogs deaminate 2-F-araAMP. Since 2-F-araAMP is more resistant to deamination than is araA, it might be anticipated that the cytotoxic dose of 2-F-araAMP for humans will be greater than the cytotoxic dose of 40 mg/m^2 observed for dogs.

The cleavage of 2-F-araAMP to 2-F-A may have clinical significance in view of the observed cytotoxicity of 2-F-A against H.Ep. 2 cells in culture (22). In mice administered a single ip dose of 2-F-A, an LD10 of 11 mg/kg has been determined (F. M. Schabel, Jr, unpublished results). Additionally, the accumulation of 2-F-A in the kidneys and bladder of dogs administered therapeutic doses of 2-F-araA has been implicated in the development of bladder toxicity in these animals (14). The toxicity was characterized by epithelial vacuolar degeneration and extensive exfoliation of this tissue; moderate submucosal edema with scattered, small hemorrhagic foci were observed. Presumably, the accumulation of 2-F-A in bladder and kidneys could be controlled by hydration and diuresis. These steps may also be advantageous during 2-F-araAMP administration to prevent the precipitation of 2-F-araA, which is also highly insoluble, in these tissues.

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Whole-Body Autoradiographic Study of the Distribution of ^{195m}Pt in Healthy and Tumor-Bearing Mice Treated With Labeled Cisplatin[1]

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^{195m}Pt-labeled cisplatin was administered iv and ip to control mice and to mice bearing Sarcoma 180. The delivered dose was 8 mg/kg and the injected activity was 80 μCi/kg. Whole-body autoradiography was performed at various times after treatment. For all animals, concentration of radioactive material was observed in the kidneys as early as 5 minutes after treatment. Most of the delivered radioactivity was present in the urine-bladder for the first 4 hours after the injection. Autoradiography also showed concentration of ^{195m}Pt in the bile ducts. These results suggest that a lower but significant part of the delivered radioactivity was also eliminated in the bile, which may explain in part the presence of the isotope in the gut lumen. In addition, ^{195m}Pt-equivalents were observed in the kidney parenchyma, the cartilages, the teeth, the skin, and the hair. Only trace amounts of ^{195m}Pt were present in the hypophysis and the testes. The distribution of radioisotope in healthy and tumor-bearing animals was comparable. Measurable uptake occurred in the Sarcoma 180 tumor. [Cancer Treat Rep 67:457–466, 1983]

Rosenberg and co-workers (1) first demonstrated the antitumor activity of cisplatin (DDP) in experimental tumor-bearing animals (2). It is now rather well established that this drug is also effective alone or in combination for the treatment of a wide variety of solid cancers in man (3). It has been shown to arrest gonadal cancers (testis and ovary) and in addition it is palliative in the treatment of several other cancers (prostate, lung, urinary bladder, and head and neck) (for a review, *see* ref 4).

Many pharmacokinetic studies have been performed with DDP in animals and in man (5). However, very few studies dealing with the distribution of this drug in the body have been reported. Whole-body scanning of humans exposed to labeled DDP was used to follow the decrease of radioactivity in the liver (6), but these studies did not detect the specific distribution of DDP within organs. Gamma camera pictures of rats treated with labeled DDP have been published (7), but they had very poor resolution so that the kinetics of radioactivity accumulation could be followed only in the bladder. A similar study in rabbits was also reported (8). Autoradiographic studies permit the exact localization of radioactivity in all the organs (9). One large study was previously published (10) in which whole-body autoradiography was performed. Unfortunately, only three animals were used, and they were killed 1, 2, and 3 hours after DDP treatment. This paper describes the whole-body autoradiography of mice that were killed between 5 minutes and 5 days after treatment with ^{195m}Pt-labeled radioactive DDP. The results permit the localization of radioactive platinum within the organs, visualization of platinum loss, and determination of the route of its elimination.

METHODS

The study was performed with DDP labeled with the ^{195m}Pt radionuclide (11) chosen because of its energy. The half-life of this gamma emitter is 4.02 days (12).

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DDP was synthesized by oxidation of ^{195m}Pt (Pt^{0}) to $H_2[PtCl_6]$ (Pt^{IV}) (13) followed by reduction to $K_2[PtCl_4]$ (Pt^{II}) (14), with the last step performed according to Dhara's method (15). The isotopic purity of ^{195m}Pt was found to be > 95%. The radiochemical purity of ^{195m}Pt-DDP was tested by radiochromatography, and the specific activity of the labeled compound thus obtained was 10.5 $\mu Ci.mg^{-1}$. The labeled preparation was dissolved in NaCl (0.9% solution).

Male and female C57B1 and Swiss mice weighing 20–25 g were caged separately and divided into two groups. Mice in Group 1 were control animals and mice in Group 2 were tumor-bearing animals. The tumor model used was Sarcoma 180, which is sensitive to DDP treatment (2). The solid tumors were obtained by injecting a suspension of 10^6 Sarcoma 180 cells sc in the axillary region of Swiss mice. Five days after graft, a solid tumor was present at the site of injection. This tumor was confirmed by necropsy and pathologic studies on some animals. Seven days after tumor implantation, the animals were injected either iv (caudal tail vein) or ip (peritoneum) with 8 mg/kg of ^{195m}Pt-labeled DDP; the delivered radioactivity was 80 $\mu Ci/kg$. Two animals from each group (iv and ip) were killed at each time point.

Animals were killed 5 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 7 hours, 16 hours, 24 hours, 48 hours, 4 days, or 5 days after treatment and prepared for whole-body autoradiography (16). First, they were frozen by rapid immersion in a freezing medium composed of methanol and dry ice. They were then embedded in a 10% aqueous solution of carboxymethylcellulose and frozen to form a firm support all around the carcass. The frozen animals were cut sagittally until an appropriate section appeared. Whole-body sagittal sections were then obtained either with a Leitz 1300 K cryomicrotome or with a PMV 450 semiautomatic microtome. Before each section was collected, adhesive tape was fixed on the surface of the animal to be sectioned. Then the microtome stage was moved and a 20-μm thick section was obtained on the adhesive tape. After the sections were collected, they were dried by storage overnight at –20°C in a cryostat. The dried sections were then pressed directly against a single-coated x-ray film (Kodak Kodirex) in a lighttight bag and stored at –30°C. At the end of a 3-week exposure period, the section and the film were separated. The former was stained when necessary with Mayer's hematoxylin for histologic examination; the latter was developed, fixed, and rinsed. All of these operations were performed in well-defined, identical conditions to produce autoradiograms that could be compared.

Moreover, sections were obtained from animals injected with unlabeled DDP. These control sections were prepared by the same procedure and pressed against Kodak Kodirex x-ray film in order to verify that no positive or negative chemography occurs with this film under the conditions of our experiment.

RESULTS

Control sections obtained from tumor-bearing and non-tumor-bearing animals treated with unlabeled DDP did not reveal any positive or negative chemography. Distribution of radioactivity in the body was very similar in non-tumor-bearing and tumor-bearing mice treated either iv (table 1) or ip with ^{195m}Pt-DDP.

Blood.—The radioactivity in blood 5 minutes after iv administration was rather concentrated. As shown in figure 1, except for the kidney, the highest concentration of radioactivity was present in cardiac cavities and in the hepatic blood vessels. The blood radioactivity persisted at least 30 minutes. Then it decreased quickly to trace amounts which were still present in animals killed 16 hours after treatment.

Kidney.—Autoradiograms of animals killed 5 minutes after iv administration (fig 1) showed that the kidney was the only organ which concentrated large amounts of radioactive material distributed heterogeneously. The parenchyma fixed a large amount of radioactivity, and by studying enlargements of autoradiograms it was possible to see that the highest concentration of radioactive material was present in the tubules. Concomitantly, a very low activity was detected in the blood of kidney vessels (fig 2). The absolute amount of radioactivity in the kidney decreased with time after injection. However, the relative concentration in the kidney was always greater than elsewhere in the body. Radioactivity was still observed in the kidney 5 days after treatment.

Liver.—Five minutes after iv or ip injection, the ^{195m}Pt concentration in the liver was lower than in the kidney. In animals killed 1 hour after treatment, an increase of radioactivity was observed in this organ and persisted as long as 5 days after treatment. In several animals it was possible to see that the gall bladder fixed some radioactive material (fig 3). Radioactivity in bile was still detectable 24 hours after iv treatment.

Bones.—^{195m}Pt-equivalents were observed in the bones of all the animals examined. In all cases, there was some radioactivity found in the bone marrow but the highest concentrations were present in cartilage (fig 4). Appreciable amounts of ^{195m}Pt were observed in all the cartilage of the body, including that of the trachea (fig 5). In animals killed between 2 and 5 days after iv or ip treatment, the concentrations of ^{195m}Pt were very low in the bone compared to the cartilage.

Teeth.—Small concentrations of radioactive material were observed in teeth of some animals in the first hours after iv administration (fig 6).

Tumor.—A low significant radioactivity was detected in this tissue (fig 7), with a heterogeneous distribution.

TABLE 1.-Distribution of radioactivity in the body of mice injected iv with labeled DDP as studied by whole-body autoradiography *

		5 min	30 min	1 hr	2 hr	4 hr	7 hr	16 hr	24 hr	48 hr	4 d
BRAIN	C	0	0	+	(+)	(+)	0	0	0	0	0
	T	0	0	+	(+)	(+)	0	0	0	0	0
BLOOD	C	+	+	(+)	(+)	(+)	(+)	0	0	0	0
	T	+	+	(+)	(+)	(+)	(+)	(+)	0	0	0
LUNGS	C	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	T	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
STOMACH CONTENT	C	0	0	0	++	+	(+)	0	0	0	0
	T	0	0	0	(+)	(+)	(+)	0	0	0	0
INTESTINAL CONTENT	C	+	+	+	++	+++	++++	(+)	(+)	(+)	0
	T	0	+	+	+++	+++	++++	0	0	0	0
LIVER	C	++	+	++	++	++	+(+)	+(+)	(+)	+	(+)
	T	++	++	++	++	++	+(+)	+(+)	+	(+)	(+)
GALL BLADDER	C	0	0	+	+	++	++	+	(+)	0	0
	T	(+)	++	++	++(+)	++(+)	++(+)	(+)	(+)	0	0
KIDNEY PARENCHYMA	C	++++	++	++	++	++	++	++	++	++	+
	T	++++	+++	+++	+++	+++	+++	++	++	++	++
KIDNEY PELVIS	C	+++++	+++++	++++	+	(+)	0	0	0	0	0
	T	+++++	+++++	+++	+	+	0	0	0	0	0
BLADDER	C	+++++	+++++	+++++	++++	++++	+++	(+)	(+)	(+)	0
	T	+++++	+++++	++++	+++++	++++	++	++	0	0	0
SPLEEN	C	+	+	(+)	(+)	(+)	(+)	(+)	0	0	0
	T	+	+	(+)	(+)	(+)	(+)	(+)	0	0	0
BONE	C	(+)	+	+	+	+	+	+	+	+	(+)
	T	(+)	+	+	++	++	++	+	+	+	+
SKIN AND HAIR	C	++	++	++	+(+)	+	+	+	+	+	(+)
	T	++	++	++	+(+)	+(+)	++	+	+	(+)	(+)
EYES	C	+	+	(+)	(+)	(+)	(+)	(+)	(+)	0	0
	T	+	+	+	(+)	(+)	(+)	(+)	(+)	0	0
THYMUS GLAND	C	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	T	+	+	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)
CARTILAGE	C	++	+	+	+	++	++	++	++	++	+
	T	++(+)	+++	+++	+++	++(+)	++(+)	++	+(+)	(+)	(+)
TUMOR	C	-	-	-	-	-	-	-	-	-	-
	T	++	++	++	++	++	+(+)	+(+)	+(+)	+(+)	+(+)
TESTES	C	+	+	(+)	(+)	(+)	(+)	0	0	0	0
	T	-	-	-	-	-	-	-	-	-	-

* C = control animals ; T = Tumor bearing animals ;

0, No radioactivity ; +++++, very high radioactivity

$0 < (+) < + < +(+) < ++ < +++ < ++++ < +++++$

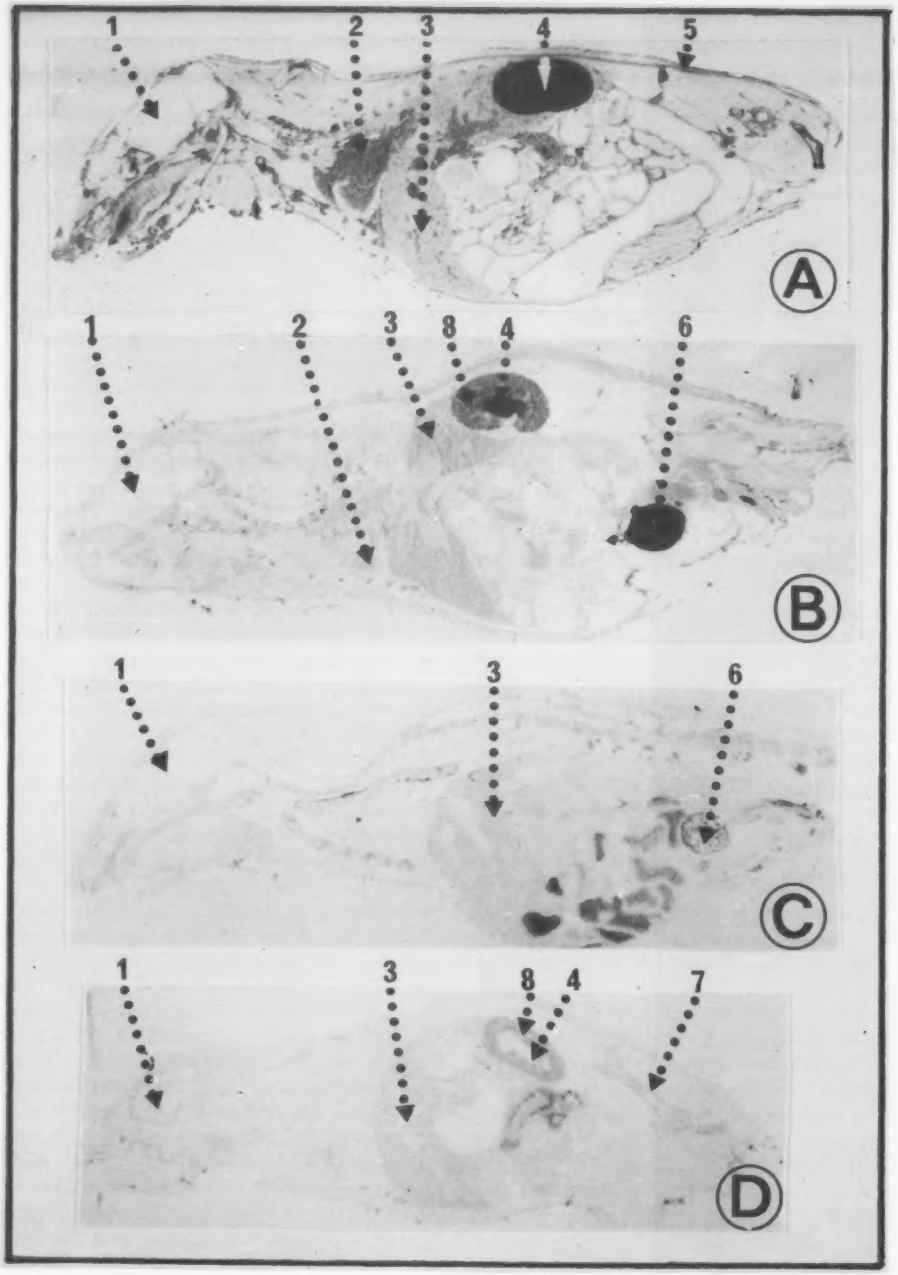

FIGURE 1.—Whole-body autoradiograms of mice after iv injection of ^{195m}Pt-DDP. Dark areas correspond to radioactivity. A, 5 mins; B, 30 mins; C, 4 hrs; D, 24 hrs. 1, brain; 2, blood; 3, liver; 4, kidney pelvis; 5, skin; 6, urinary bladder; 7, pelvic bone; 8, kidney parenchyma.

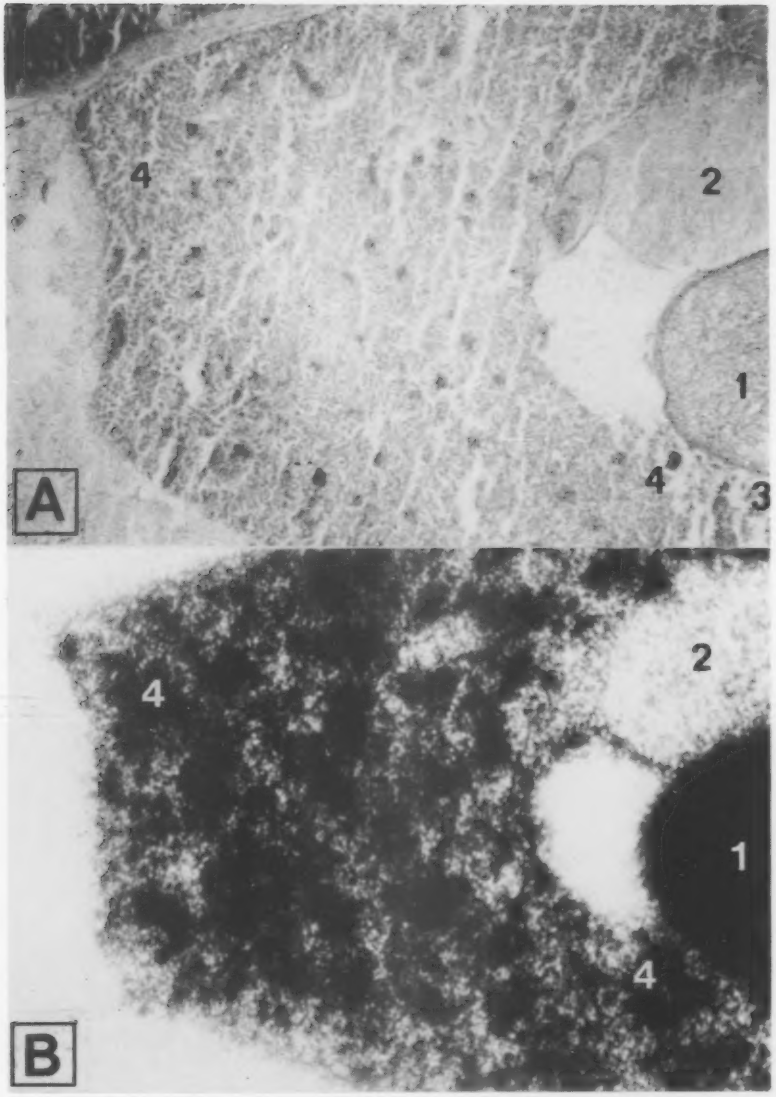

FIGURE 2.—Microscopy examination of a whole-body section of a mouse injected iv with ^{195m}Pt-DDP and killed 5 mins later (× 25). Details of the kidney: A, stained section; B, autoradiogram. 1, pelvis; 2, blood; 3, pyramid; 4, tubules.

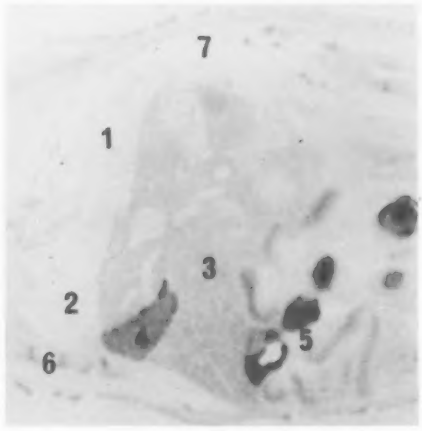

FIGURE 3.—Details of an autoradiogram of a mouse injected iv with ^{195m}Pt-DDP and killed 4 hrs later. Note uptake in the gallbladder. 1, lungs; 2, heart blood; 3, liver; 4, gallbladder; 5, intestines; 6, sternum; 7, muscles.

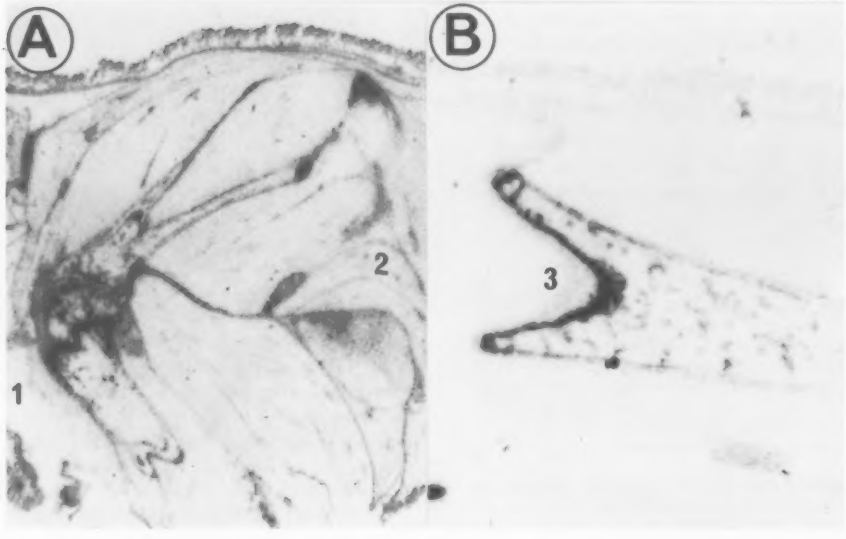

FIGURE 4.—Details of autoradiograms of mice injected iv (A) and ip (B) with ^{195m}Pt-DDP and killed 5 mins (A) and 4 hrs (B) later. Note incorporation of ^{195m}Pt in the epiphyseal cartilage of the humerus (1), the cartilage of the scapula (2), and the cartilage of the pelvic bone (3).

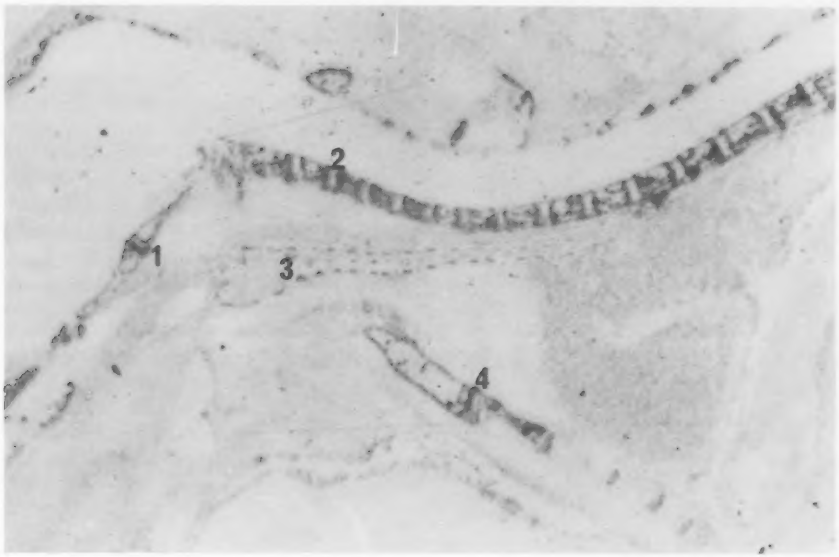

FIGURE 5.—Enlargement of a whole-body autoradiogram of a mouse injected ip with ^{195m}Pt-DDP and killed 2 hrs later. Note uptake in cartilage of skull (1), vertebrae (2), trachea (3), and sternum (4).

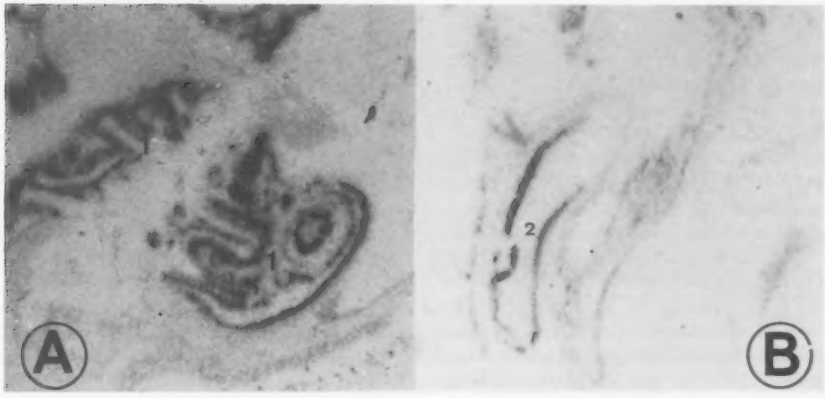

FIGURE 6.—Details of autoradiograms of mice injected iv (A) and ip (B) with ^{195m}Pt-DDP and killed 2 hrs (A) and 7 hrs (B) later. Note fixation on molar teeth (1) and incisor tooth (2).

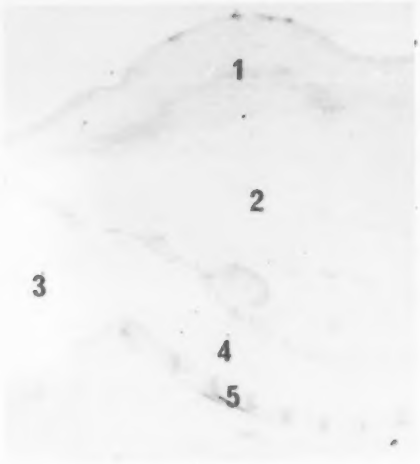

FIGURE 7.—Details of an autoradiogram of a mouse injected ip with ^{195m}Pt-DDP and killed 30 mins later showing the tumor. 1, tumor; 2, muscles; 3, brain; 4, spinal cord; 5, cervical vertebrae.

Miscellaneous.—Some other particular localizations were observed. Persistent activity was present in the skin and in the distal regions of hair (fig 8). In some animals, it was also possible to detect rather weak activity in the hypophysis and the testes.

DISCUSSION

The results presented in this paper give an idea of the distribution of ^{195m}Pt-equivalents in the mouse body, the elimination pathways, and the organs in which the isotope is fixed.

In the first hour after DDP administration, there is a rapid decrease of radioactivity in the bloodstream, followed by a slow loss of radioactivity during the next 16 hours. These results agree with the well-documented conclusion that the plasma clearance of platinum after a single dose of DDP is biphasic, with a rapid component characterized by a half-life on the order of minutes and a terminal phase characterized by a half-life on the order of days (5). This conclusion has been reported in studies of different animal species (7,11,17–21).

Most of the radioactivity delivered to mice is excreted in urine. This is demonstrated by the very high uptake of ^{195m}Pt-equivalents in the kidney as early as 5 minutes after iv or ip administration and an intense incorporation of radioactivity in urine in both the ureter and the bladder (fig 1). It is worth mentioning that a high intestinal content at 4–7 hours, which rapidly fell to zero by 16 hours, could be related to biliary elimination. To our knowledge, few reports deal with biliary excretion of DDP. In the female dogfish shark, platinum is found in the bile 6 days after treatment (18). In the dog, small amounts of platinum have been detected in bile following an iv dose of DDP (17); in man, concentrations in the bile reached 15% of the highest plasma level after a rapid infusion and 8% of the highest plasma level after a 6-hour infusion (21).

Our results indicate the high affinity of platinum for the kidney when DDP is delivered to animals; this may be in part responsible for the nephrotoxicity, ie, the main toxicity, of this compound (22–30). Ultrastructural kidney damage concerns mainly proximal tubules (31). Hence, kidney damage may be correlated with the observed concentration of radioactive platinum in tubules (fig 2).

In all animals studied we noticed an uptake of radioactive material in the liver. Pharmacokinetic studies have shown a similar uptake in the liver in man (6,32) and in animals (10). However, liver toxicity has been reported in only one clinical study (33).

The persistence of radioactivity in bone marrow may be correlated with the toxic effects of DDP on the hematopoietic organs (eg, bone marrow aplasia, splenic atrophy, and atrophy of lymph nodes and thymus) (30).

We observed a concentration of ^{195m}Pt in the teeth of several animals, which has not been previously observed. However, it has recently been reported that a child regularly treated with DDP developed a dark line on the teeth (34) which was comparable to gingival lines characteristic of human intoxication with other heavy metals.

Platinum was also observed in the skin and hair of animals treated with DDP; to our knowledge, this has not been previously reported. Animals implanted with Sarcoma 180 appeared to concentrate ^{195m}Pt in the tumor. This uptake was measured and found to be between 0.5% and 1.5% of the delivered dose (35). The same range of concentration was observed (36) under different experimental conditions with an ascitic tumor, leukemia L1210, in DBA/2 mice. In contrast, tumor-bearing rats implanted with the BICR/A3 osteogenic sarcoma or the BICR/M2 fibrosarcoma incorporated relatively little platinum in tumor tissues, and the rate of clearance was more rapid from tumors than from kidney or liver (37). No specific uptake by the tumor was reported for rats implanted with the Walker 256 carcinosarcoma (10).

In conclusion, this autoradiographic study of ^{195m}Pt in mice has shown that DDP is eliminated primarily in the urine and to a lesser extent through the bile. Organ distribution of the labeled compound can be correlated with some toxic effects of this drug. Whole-body autoradiography seems a promising technique for the study of distribution, elimination pathways, and organ specificity of radiolabeled new platinum antitumor compounds.

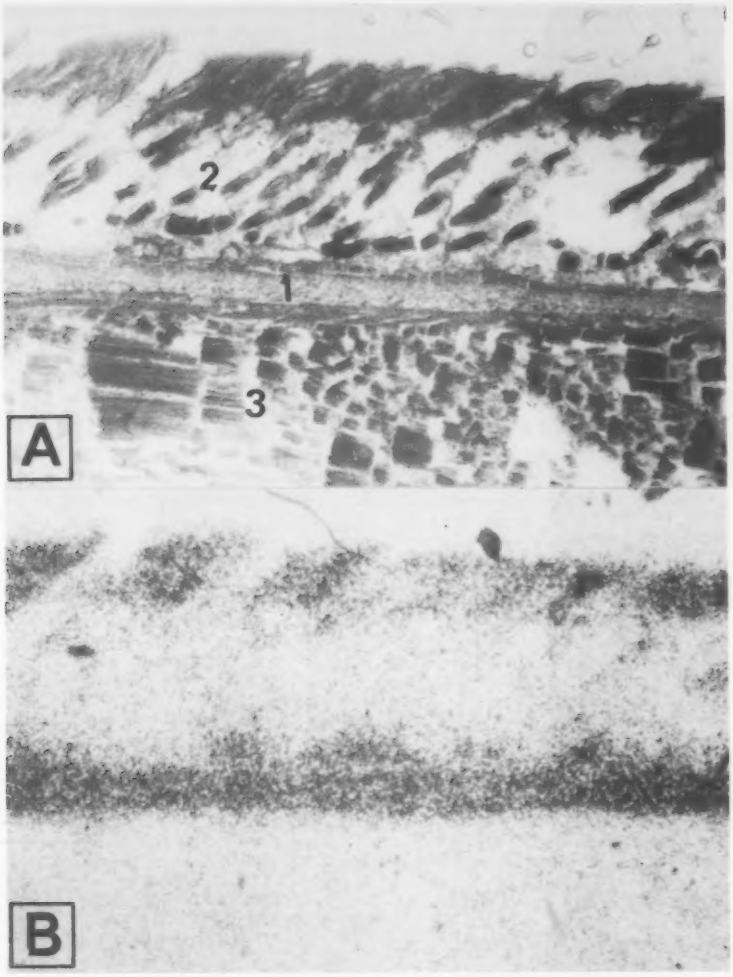

FIGURE 8.—Enlargement of an autoradiogram of a mouse injected ip with ^{195m}Pt-DDP and killed 4 hrs later. Note uptake in the skin. 1, epidermis; 2, hair; 3, muscle. A, stained section; B, autoradiogram (× 25).

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Effects of Amsacrine (*m*-AMSA), a New Aminoacridine Antitumor Drug, on the Rabbit Heart[1,2]

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There is emerging clinical evidence that amsacrine (*m*-AMSA) administration may be associated with cardiotoxic effects such as severe, even fatal, ventricular arrhythmias and impairment of the inotropic performance of the heart. Information on the cardiac effects of *m*-AMSA in animals is scanty. Studies on mice, dogs, and monkeys have not evidenced the cardiotoxicity of the compound. The data presented in this paper show that *m*-AMSA causes acute ECG alterations in normal rabbits and a dose-related negative inotropic effect on the isolated rabbit heart, suggesting that this species may be a useful model for the study of the cardiac actions of this antiblastic. [Cancer Treat Rep 67:467–474, 1983]

Amsacrine (*m*-AMSA) is a new antitumor drug with an aminoacridine structure. In the clinical trials to date, the molecule has shown activity mainly against acute leukemias and lymphomas (1). The predominant side effect of the drug is a dose-related leukopenia (1). However, some clinical reports have called attention to serious cardiac effects induced by this antiblastic, such as ventricular arrhythmias (2–4) and, in individuals with previous exposure to anthracyclines, echocardiographically documented impairment of the inotropic performance of the myocardium (5–7). The mechanism of these disturbances is unknown, even though it has been suggested that electrolyte imbalances (such as hypokalemia) and the effects of the vehicle for *m*-AMSA (*N*,*N*-dimethylacetamide and lactic acid) may contribute to the genesis of the rhythm alterations (3).

Because studies on mice, dogs, and monkeys (8) have not evidenced any cardiotoxicity of the aminoacridine, we decided to investigate the influence of *m*-AMSA on the rabbit heart, with the aim of finding an animal model suitable for the study of both the mechanism and the possible antagonistic modalities of its cardiac actions. We chose the rabbit because it has been shown to be a good model of anthracycline cardiotoxicity (9,10).

MATERIALS AND METHODS

m-AMSA was supplied by Mr. L. H. Kedda, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The formulation of the drug was identical to that in clinical use. *m*-AMSA (50 mg) was dissolved in 1 ml of anhydrous *N*,*N*-dimethylacetamide, and this solution was added to 9 ml of 0.0353 M L-lactic acid to yield a solution containing 5 mg/ml of *m*-AMSA, 2.8 mg/ml of L-lactic acid, and 100 mg/ml of *N*,*N*-dimethylacetamide, pH 3.5. Vehicle solutions contained equivalent amounts of *N*,*N*-dimethylacetamide and L-lactic acid, pH 3.2. The solutions were prepared immediately before use.

Local male rabbits (1.8–2.5 kg) were used.

In Vivo Studies

Unanesthetized rabbits were treated by rapid iv injection (ear veins) with different doses of *m*-AMSA or of its vehicle solution. ECGs were recorded for at least 1 hour following administration, by means of needle electrodes inserted into the limbs. The survival time of the animals was checked and drug-related deaths were

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assumed to be those occurring within 30 days after the injection.

In Vitro Studies

Animals were treated iv with 500 units/kg of sodium heparin (Istituto Chemioterapico Italiano, Milan, Italy). Twenty minutes later they were stunned by a blow to the base of the skull. The heart was rapidly excised and, after removal of pericardial tissue, was gently washed in Locke-Ringer solution and mounted, by means of aortic root, in a Langendorff apparatus. The heart was perfused in retrograde manner, at constant pressure (45 cm of water) and at constant temperature (37.5°C), with an oxygenated Locke-Ringer solution of the following composition (mmol): NaCl, 49.9; KCl, 5.63; $CaCl_2$, 2.52; $NaHCO_3$, 3.57; and glucose, 5.63 mM. The pH of the perfusion fluid was 7.4. Left ventricular pressure (LVP) was measured by means of a water-filled balloon inserted into the left ventricle through a cut in the left atrial appendage; the balloon was connected to a pressure transducer by a stiff polyethylene catheter. The first derivative of LVP (dP/dt_{max}) was obtained by means of a differentiator module (O.T.E. Biomedica Module 1087). LVP and dP/dt_{max} were recorded on a polygraph (O.T.E. Biomedica). Coronary flow was measured by collecting the fluid draining from the heart. Heart rate was maintained 60 beats over basal values by electric stimulation by means of two electrodes inserted into the right atrium and in the ventricular apex, respectively. A stimulus of 10 V with duration of 5 milliseconds (CF Palmer Stimulator Model 8048) was applied: this provided a constant rate for each heart in the range of about 250 beats/minute.

Hearts were perfused for 1 hour before drug addition to obtain stabilization of heart contractions and therefore basal values of the parameters to be evaluated. *m*-AMSA was given at the concentrations shown in table 1, dissolved in perfusion fluid; pH value of the perfusion fluid was not altered by the drug. Each concentration of the drug was tested on different groups of five animals.

Statistical Analysis

The effects of *m*-AMSA at given concentrations on LVP, dP/dt_{max}, and coronary flow were analyzed by Student's t test for paired samples assuming the 0.05 level of probability as significant.

The dose-response relationship was obtained by means of linear regression analysis; the probability of significant regression was computed by analysis of variance.

RESULTS

In Vivo Studies

A number of ECG abnormalities appeared soon after the administration of *m*-AMSA (table 2 and figs 1–4), occurring more frequently in doses ≥ 4 mg/kg; the 4-mg/kg dose was fatal in two of the five animals treated. Altered impulse conduction at the level of the atrioventricular (AV) node and/or the His bundle and branches was noted (as first- or second-degree AV block or widening of the QRS complexes of the branch block type or QRS complex alterations of left anterior or posterior hemiblock type) at each dose level in 11 rabbits (Nos. 4, 5, 8, 10, 13, 14, 18, 19, 20, 22, and 23) of the 17 that had rhythm abnormalities. In three other rabbits (Nos. 9, 15, and 17), we also observed a widening of the QRS complexes that nevertheless was probably due to the presence of supraventricular tachycardia. Ectopic impulse generation probably caused supraventricular arrhythmias (flutter, tachycardia) in four rabbits (Nos. 9, 15, 17, and 21) and ventricular arrhythmias (extrasystoles, tachycardia) in eight (Nos. 7, 9, 12, 13, 15, 18, 19, and 21). The administration of the *m*-AMSA vehicle, within a range of volumes equivalent to those used to administer the compound, resulted in no ECG alteration.

TABLE 1.—Effects of *m*-AMSA at given concentrations on LVP, dP/dt_{max}, and coronary flow*

Concentration (M)	LVP (% change)	*P* value	dP/dt_{max} (% change)	*P* value	Coronary flow (% change)	*P* value
			During m-AMSA perfusion			
6.3×10^{-6}	−48.4 ± 6.9	< 0.01	−65.8 ± 17	< 0.01	−18.3 ± 7.1	Not significant
3.8×10^{-6}	−23.8 ± 13.9	< 0.02	−49.6 ± 6.6	< 0.02	−18.2 ± 13.3	Not significant
2.5×10^{-6}	−18 ± 9.7	Not significant	−32 ± 6.7	< 0.05	−17.6 ± 23	Not significant
1.2×10^{-6}	−14 ± 3.3	< 0.02	−28.5 ± 9.1	< 0.05	−16.6 ± 4.1	Not significant
			After stopping m-AMSA perfusion			
6.3×10^{-6}	−7.2 ± 9.7	Not significant	− 9.3 ± 12.1	Not significant	− 7.4 ± 12	Not significant
3.8×10^{-6}	−9 ± 3.4	Not significant	−14.9 ± 16.2	Not significant	−19.6 ± 13.7	Not significant
2.5×10^{-6}	−2.8 ± 4.2	Not significant	− 1.8 ± 14.2	Not significant	− 7 ± 22.3	Not significant
1.2×10^{-6}	−3.4 ± 3.1	Not significant	− 3 ± 13.7	Not significant	−11.5 ± 9.3	Not significant

*Values = mean ± SE of 5 experiments. For all concentrations different groups of animals were used. Data were analyzed by Student's t test for paired samples, comparing both series of data (during and after perfusion) with basal values.

TABLE 2.—ECG changes and survival time of rabbits treated with *m*-AMSA or its vehicle solution

Rabbit No.	Treatment (drug, schedule, route)	ECG change	Time of death after treatment*
1	*m*-AMSA, 2.5 mg/kg, iv	No modification	S
2	*m*-AMSA, 2.5 mg/kg, iv	No modification	S
3	*m*-AMSA, 2.5 mg/kg, iv	No modification	S
4	*m*-AMSA, 2.5 mg/kg, iv	Immediately after *m*-AMSA, modification of the QRS axis of left posterior hemiblock type which persisted over 30 mins	S
5	*m*-AMSA, 2.5 mg/kg, iv	Immediately after *m*-AMSA, modification of the QRS axis of left posterior hemiblock type and prolongation of the P-Q interval (1st-degree AV block) which persisted over 10 mins	S
6	*m*-AMSA, 4 mg/kg, iv	No modification	Dead after 20 days
7	*m*-AMSA, 4 mg/kg, iv	Trigeminal ventricular extrasystoles from 3 to 5 mins after *m*-AMSA	Dead after 23 days
8	*m*-AMSA, 4 mg/kg, iv	Modification of the QRS axis of left posterior hemiblock type from 2 to 12 mins after *m*-AMSA	S
9	*m*-AMSA, 4 mg/kg, iv	Immediately after *m*-AMSA, supraventricular tachycardia with widened QRS complexes followed by ventricular tachycardia which regressed at 3 mins after administration	S
10	*m*-AMSA, 4 mg/kg, iv	Immediately after *m*-AMSA, modification of the QRS axis of left anterior hemiblock type and sporadic episodes of 2:1 AV block; the alterations regressed at 3 mins	S
11	*m*-AMSA, 5 mg/kg, iv	No modification	S
12	*m*-AMSA, 5 mg/kg, iv	Frequent ventricular extrasystoles from 3 to 15 mins after *m*-AMSA	S
13	*m*-AMSA, 5 mg/kg, iv	Prolongation of the P-Q interval and widening of the QRS complexes from 1 to 5 mins after *m*-AMSA; frequent ventricular extrasystoles from 13 to 20 mins after *m*-AMSA	Dead after 27 days
14	*m*-AMSA, 5 mg/kg, iv	Immediately after *m*-AMSA, widening of the QRS complexes of intraventricular conduction alteration type	Dead after 4 days
15	*m*-AMSA, 5 mg/kg, iv	1 min after *m*-AMSA, atrial flutter with variable AV conduction and aberrant intraventricular conduction; polyfocal ventricular arrhythmias	Dead after 5 days
16	*m*-AMSA, 7.5 mg/kg, iv	No modification	S
17	*m*-AMSA, 7.5 mg/kg, iv	Immediately after *m*-AMSA, supraventricular tachycardia with aberrant conduction which persisted for 3 mins	Dead after 7 days
18	*m*-AMSA, 7.5 mg/kg, iv	Immediately after *m*-AMSA, widening of the QRS complexes followed by ventricular tachycardia; at 4 mins, 2nd-degree AV block with 2:1 conduction; at 15 mins, 1st-degree AV block until normalization which occurred at 20 mins	Dead after 3 days
19	*m*-AMSA, 7.5 mg/kg, iv	1 min after *m*-AMSA, widening of the QRS complexes and short bursts of ventricular tachycardia; at 2 mins, conversion into 2nd-degree AV block with short bursts of supraventricular tachycardia; normalization at 7 mins	Dead after 7 days
20	*m*-AMSA, 7.5 mg/kg, iv	Immediately after *m*-AMSA, 1st-degree AV block and modification of the QRS complexes of left posterior hemiblock type; gradual regression of the alterations	Dead after 6 days
21	*m*-AMSA, 10 mg/kg, iv	Immediately after *m*-AMSA, ventricular tachycardia; after 3 mins, atrial flutter with slightly widened QRS complexes that regressed at 7 mins	Dead after 3 days

TABLE 2.—Continued

Rabbit No.	Treatment (drug, schedule, route)	ECG change	Time of death after treatment*
22	*m*-AMSA, 15 mg/kg, iv	From 2 to 5 mins after *m*-AMSA, 2nd-degree AV block with widened QRS complexes and short bursts of ventricular tachycardia; from 10 to 60 mins, sinus tachycardia	Dead after 5 days
23	*m*-AMSA, 15 mg/kg, iv	1.5 mins after *m*-AMSA, 1st-degree AV block with variable P-Q intervals and periods of 2nd-grade (II type) AV block; widened QRS complexes and frequent supraventricular extrasystoles; at 4.5 mins, atrial tachycardia with 2nd-degree AV block was present until normalization which occurred at 10 mins	Dead after 5 days
24	Vehicle solution, 1.5 ml/kg, iv (as *m*-AMSA, 7.5 mg/kg)	No modification	S
25	Vehicle solution, 1.5 ml/kg, iv (as *m*-AMSA, 7.5 mg/kg)	No modification	S
26	Vehicle solution, 2 ml/kg, iv (as *m*-AMSA, 10 mg/kg)	No modification	S
27	Vehicle solution, 2 ml/kg, iv (as *m*-AMSA, 10 mg/kg)	No modification	S
28	Vehicle solution 3 ml/kg, iv (as *m*-AMSA, 15 mg/kg)	No modification	S

*S = surviving 30 days after treatment.

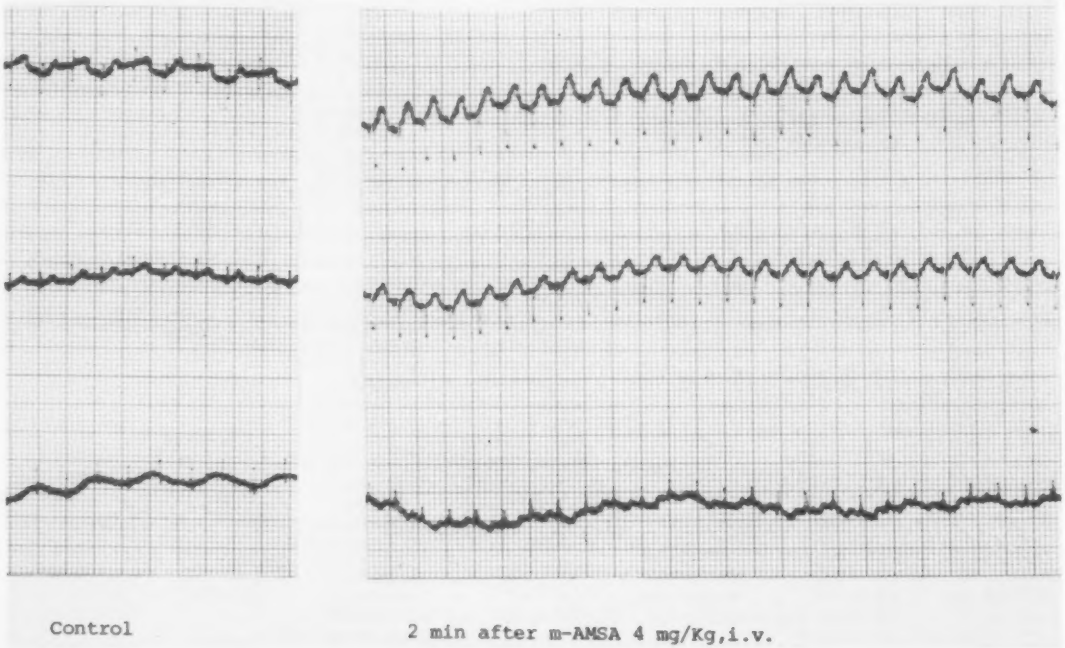

FIGURE 1.—ECG of Rabbit 8. Standard leads I, II, and III (25 mm/sec).

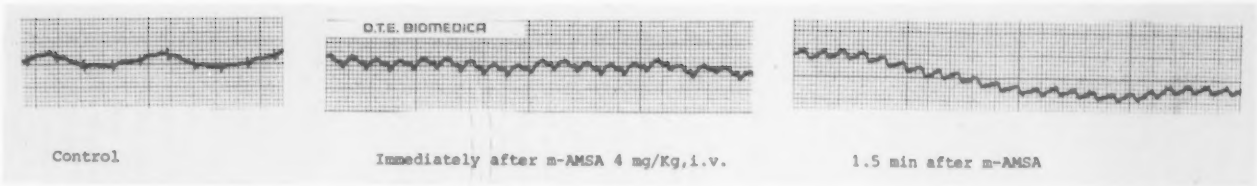

FIGURE 2.—ECG of Rabbit 9. Standard lead II (25 mm/sec).

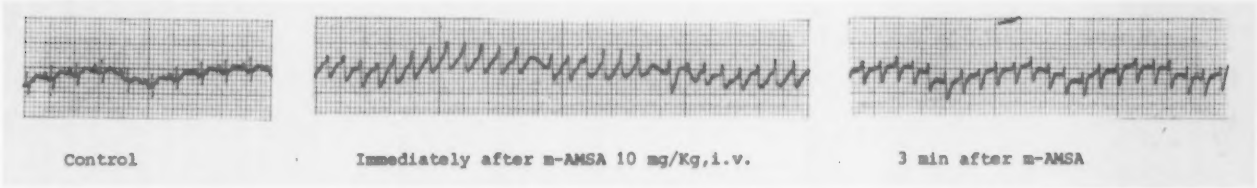

FIGURE 3.—ECG of Rabbit 21. Standard lead III (25 mm/sec).

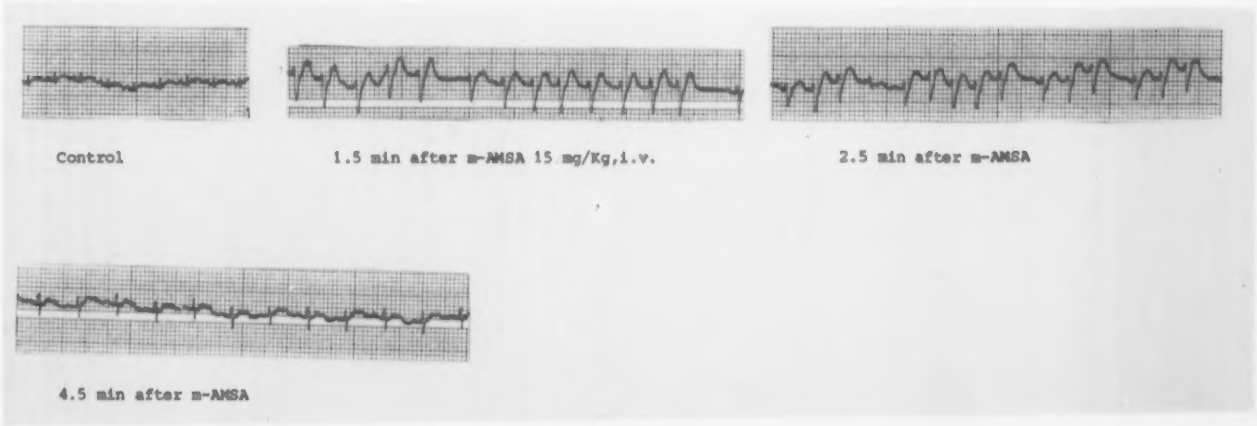

FIGURE 4.—ECG of Rabbit 23. Standard lead III (25 mm/sec).

In Vitro Studies

In electrically paced isolated rabbit hearts *m*-AMSA caused a dose-related decrease of both LVP and dP/dt_{max} (table 1; figs 5 and 6). No statistically significant effects on coronary flow were seen. Decrease of both parameters began within 30–60 seconds from *m*-AMSA administration and stabilized on a plateau within 1–3 minutes (fig 7). Results reported in table 1 and figures 5 and 6 refer to this phase of experiments. Some minutes later, hearts escaped from electrical pacing, showing abnormalities in contractions such as depicted in figure 7. Interruption of *m*-AMSA administration at this phase of the experiments caused a return to electrical pacing and a recovery in inotropism within 10–15 minutes; sometimes a slight and not statistically significant reduction of dP/dt_{max} remained.

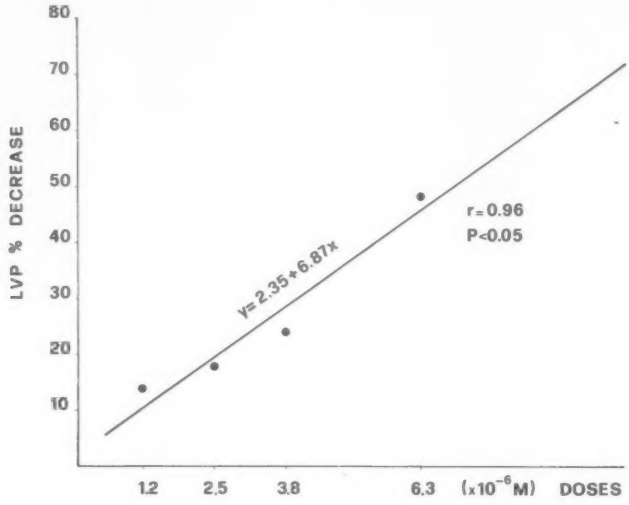

FIGURE 5.—Relation between *m*-AMSA concentrations and % decrease of LVP in paced isolated hearts. Plot was obtained from linear regression analysis.

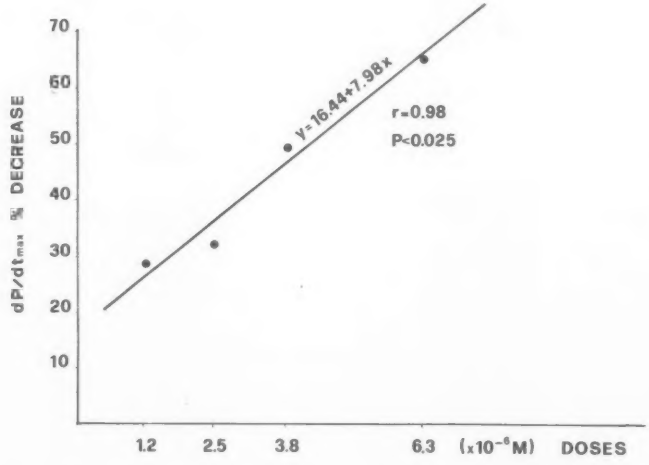

FIGURE 6.—Relation between *m*-AMSA concentrations and % decrease of dP/dt_{max} in paced isolated hearts. Plot was obtained from linear regression analysis.

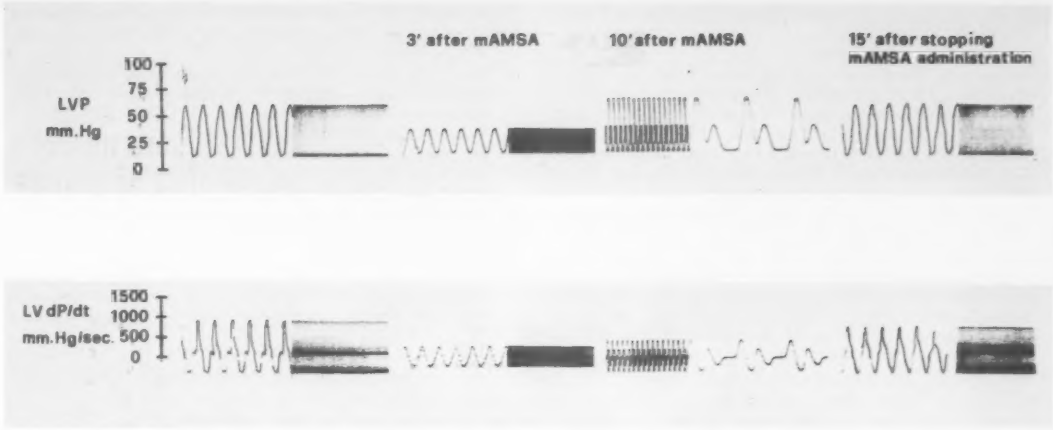

FIGURE 7.—Effect of *m*-AMSA (3.8×10^{-6} M) on paced isolated rabbit heart.

After 15 minutes of perfusion without the drug, a second and a third administration of *m*-AMSA, using the same concentration for every heart, caused very similar effects. Data obtained with a second and a third administration suggest that a short perfusion without the drug is able to remove all the drug previously bound to the heart or, at least, the fraction of the drug responsible for inotropism impairment.

Other experiments showed no effect of *m*-AMSA vehicle on heart parameters at the dose equivalent to the highest dose of *m*-AMSA. Results referring to the second and third perfusion of the drug and those obtained with *m*-AMSA vehicle are not reported for the sake of brevity.

CONCLUSIONS

Administration of *m*-AMSA to unanesthetized rabbits induced a series of cardiac rhythm abnormalities characterized by conductive alterations and/or by ectopic impulse generation. These manifestations were more frequent and severe after doses of the compound which caused death in some of the animals treated. Nevertheless, they seem specific, because in our experience comparable high doses of other noncardiotoxic antitumor agents such as mitomycin and dactinomycin did not cause such ECG changes in rabbits (unpublished observations).

The cardiac toxicity of *m*-AMSA was also evidenced by the isolated rabbit heart model where the drug, within the range of human therapeutic concentrations as reported by Van Echo et al (11), caused a dose-related negative inotropic effect. *m*-AMSA vehicle had no effect either in vitro or in vivo.

While *m*-AMSA is known to be a DNA-intercalating drug, some observations have cast doubts about whether this effect is the only important one with respect to the antitumor activity of the compound. In fact, its analog *o*-AMSA presents comparable DNA-binding properties but is devoid of any antitumor action (12). Alternatively, spin-label studies on human erythrocyte ghost membranes and murine mastocytoma cells (13) have suggested that membrane alterations may be important in the mechanism of the drug's action. This fits well with the cardiodepressive profile shown by *m*-AMSA in rabbits and humans because many membrane-active drugs produce similar effects on the electric and mechanical properties of the myocardium.

In conclusion, we think that the rabbit may represent a useful tool for the study of the cardiac actions of *m*-AMSA and for the toxicologic screening of new acridine derivatives.

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Paradoxical Behavior of 6-Mercaptopurine as a Cytotoxic Agent: Decreasing Cell Kill With Increasing Drug Dose[1,2]

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9L rat brain tumor cells were continuously exposed to "low" (2.9 μM) and "high" (18 and 35 μM) doses of 6-mercaptopurine (6-MP); cell kill was measured with a colony forming efficiency assay. The low dose of 6-MP killed more cells than the high doses, as measured by surviving fraction, when they were treated for > 48 hours. Even though the total number of cells increased during the 72-hour treatment period, the number of clonogenic cells decreased most when cells were treated with the low dose of 6-MP. Data obtained with flow cytometry suggest that for the high dose there was a drug-induced block of cells in the G_1 phase that prevented them from progression into S phase where they would be killed.
[Cancer Treat Rep 67:475–480, 1983]

Most cell cycle-specific antineoplastic agents such as 5-FU, hydroxyurea, and methotrexate kill cells preferentially at a certain phase of the cell cycle. These and other agents can also perturb cell progression, which delays DNA synthesis and blocks progression through the cell cycle at certain phases; this may produce either a synchronization or accumulation of cells at some point in the mitotic cycle. Patterns of drug-induced perturbations may depend on the amount of drug and/or the period of drug administration. While some perturbations are closely related to cytotoxic processes, this is not always the case; we have shown that nontoxic doses of 5-FU cause 9L cells to accumulate preferentially in S phase, which results in more cell kill (1).

6-Mercaptopurine (6-MP), an antimetabolite classified as a cell cycle-specific drug, has been used extensively for the treatment of various human neoplasms (2–5). We report here the results of studies that show that treating 9L monolayer cells with "low" (2.9 μM) and "high" (18 and 35 μM) doses of 6-MP perturbs progression through the cell cycle in such a way that the low dose kills more clonogenic cells than either of the high doses.

MATERIALS AND METHODS

Drug treatment.—6-MP (Calbiochem, La Jolla, CA) was dissolved in DMSO and diluted to various concentrations with Eagle's minimum essential medium (MEM). (The final concentration of DMSO in medium was 0.1%, which does not affect either cell kill or progression of cells.) Approximately 1×10^6 monolayer 9L rat brain tumor cells were seeded into 75-cm^2 tissue culture flasks (Falcon Plastics, Oxnard, CA) and grown in MEM supplemented with 10% newborn calf serum; 48 hours after seeding of cells, 6-MP was added at low (2.9 μM) and high (18 and 35 μM) doses. Cells were maintained at 37°C and pH 7.2–7.4 during the treatment period. For extended treatment protocols, the drug-containing medium was renewed every 24 hours. Immediately after treatment, cells were rinsed twice with MEM and trypsinized (Saline A containing 0.05% trypsin and 0.02% versene) for the cell survival assay.

Cell survival assay.—The colony forming efficiency assay has been described (6). Briefly, trypsinized cells were counted electronically (Royco counter) and appropriate numbers of cells were plated into Petri dishes

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with 5×10^4 heavily irradiated (40 Gy) 9L feeder cells, which is the optimum number for this system. Cells were incubated for 12–14 days at 37°C in a humidified 95% air:5% CO_2 atmosphere and stained with crystal violet in ethanol, and the number of colonies that formed were counted. The relative number of clonogenic cells surviving treatment was calculated as the plating efficiency (PE) times the number of cells obtained at each time point, divided by the PE times the number of cells at the beginning of treatment.

Autoradiography.—^{3}H-thymidine (^{3}H-TdR; 0.1 μCi/ml) (New England Nuclear Corp, Boston, MA; specific activity, 2 mCi/mol) was added to cells simultaneously with 6-MP for 30 minutes. Cells were washed, trypsinized, spread on slides that were dipped in 75% NTB-2 nuclear emulsion (Kodak, Rochester, NY), and exposed at 4°C for 10 days before being developed. Cells were stained with hematoxylin. The labeled population, defined as cells containing more than four grains per nucleus (the average background was less than one grain per nucleus), was determined by scoring 1000 cells from each slide.

Radioactivity of ^{3}H-TdR in cells.—A portion of the cell suspension labeled for autoradiography was counted for total cells and centrifuged at 1000 rpm for 5 minutes; the cell button was incubated in 2.5 ml of NCS solubilizer (Amersham Co, IL) for 30 minutes at 50°C. Permablend (15 ml) was added to each vial, and the level of radioactivity was counted on a Hewlett-Packard liquid scintillation counter. The relative rate of DNA synthesis (ability of cells in S phase to incorporate label during a 30-minute exposure to ^{3}H-TdR) was calculated by dividing the total radioactivity by the number of cells labeled (determined by autoradiography).

Flow cytometry.—Single-cell suspensions from untreated and treated cultures were centrifuged at 1000 rpm for 5 minutes, medium was decanted, and cells were fixed with 70% ethanol and stained with chromomycin A_3 (Calbiochem). Approximately 1×10^5 cells of each specimen were sorted on a FACS III flow cytometer (Becton-Dickinson, Sunnyvale, CA). Data were analyzed with Dean's computer program (7).

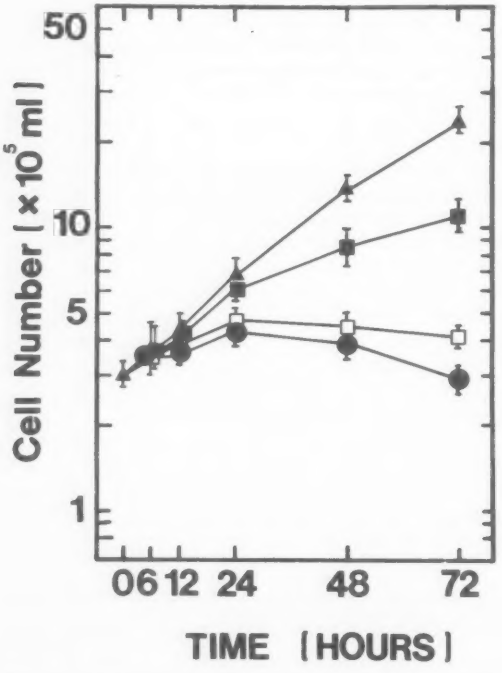

FIGURE 1.—Effect of continuous exposure to various concentrations of 6-MP. ▲, untreated; ■, 2.9 μM; □, 18 μM; and ●, 35 μM. Symbols and error bars represent the mean ± SE of 8 separate experiments.

RESULTS

Influence of 6-MP on the growth rate of 9L cells.—Figure 1 is a plot of the posttreatment growth versus time for 9L monolayer cells treated with the three doses of 6-MP. Compared to control cells, cells treated with the low dose had slower growth during 72 hours, and cells treated with the high doses proliferated at much slower rates for about 24 hours, after which the rate either did not increase or declined slightly.

Clonogenicity.—Figure 2 shows plots of surviving fraction (SF) versus time for the low and high doses. There was little difference between the SFs of cells treated with low and high concentrations of 6-MP for the first 24 hours after treatment; however, if treatment was extended beyond 24 hours, the SF of 9L cells treated with the low dose was much lower than the SFs of cells treated with the high doses. The relative number of clonogenic cells for all three drug doses decreased relatively rapidly during the 72-hour treatment period compared to controls (table 1). Relatively fewer clonogenic cells survived treatment with the low dose of 6-MP compared to cells treated at higher doses (table 1).

Analysis by flow cytometry.—Representative DNA histograms of control and treated cells, from which the distribution of G_1-, S-, and G_2/M-phase cells was determined, were obtained (not shown). Figure 3 shows that after treatment with all three doses of 6-MP for 24 hours, the percentage of G_1 cells increased gradually compared to controls. Twenty-four hours after treatment with the low dose, the percent of G_1 cells was about 64% and then it gradually decreased to the initial level for the next 48 hours. However, after treatment with the high doses, the percent of cells in G_1 phase remained high (about 70%) for the last 48 hours, while

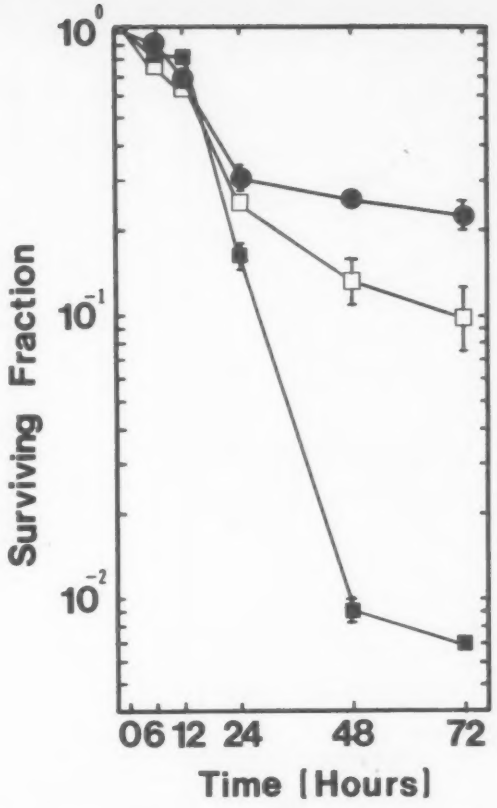

FIGURE 2.—Time-survival curves for clonogenic 9L cells after treatment with 6-MP. ■, 2.9 μM; □, 18 μM; and ●, 35 μM. Symbols and bars represent the mean ± SD from 4–8 samples from 8 separate experiments. SD is not shown if smaller than the symbols. Differences were statistically significant ($P < 0.001$) between groups for time points of 24 hrs.

the percent of cells in S phase decreased to approximately 20%.

Labeling index.—When exponentially growing 9L monolayer cells were treated simultaneously with ^{3}H-TdR and low and high doses of 6-MP, during the first 12 hours the percent of labeled cells was 41.3 ± 1.0 for control cells, 8.3 ± 2.7 for cells exposed to the low dose, and 1.8 ± 0.3 and 2.4 ± 0.5 for cells exposed to the respective high doses (table 2). There was some increase in the percent of labeled cells at 48 hours among those treated with high doses of 6-MP, although cells treated with the low dose of 6-MP decreased gradually to 2.3 ± 0.2 at 72 hours.

Relative rates of DNA synthesis.—The relative rate of DNA synthesis in 9L cells treated with low and high doses of 6-MP decreased rapidly for the first 12 hours after treatment and then returned to control levels gradually by 72 hours. Recovery of the rate of DNA synthesis was more marked for cells treated with the low dose of 6-MP than for cells treated with the high doses (fig 4); with the exception of the 72-hour point, the rate at which the relative rate increased was approximately the same for all three doses.

DISCUSSION

Both low and high doses of 6-MP are used for the treatment of systemic and solid tumors (2–5,8). 6-MP is converted intracellularly to the thio analog of inosinic acid, which is the active cytotoxic moiety that is incorporated into DNA during the S phase (9–11); 6-MP is therefore classified as a cell cycle-specific agent (12,13). However, 6-MP inhibits progression into S phase in a dose-dependent fashion (14,15), and therefore can have a self-limiting cytotoxic effect. Depending on the length of drug exposure, cells that are blocked may cycle slowly into S phase where they are killed (16).

The self-limiting effects can be more subtle, however. These studies show that a low dose (2.9 μM) of 6-MP killed more clonogenic cells than either of two high doses (18 and 35 μM). Even though the low dose of 6-MP killed more cells than the high doses, the number of cells

TABLE 1.—Relative No. of clonogenic cells at selected times after 6-MP was added to cell cultures

6-MP concentration (μM)	Relative No. of clonogenic cells* after—					
	0 hr	6 hrs	12 hrs	24 hrs	48 hrs	72 hrs
Control	1.00	1.38	1.64	2.65	6.00	11.34
2.9	1.00	0.95	0.76	0.30	0.11	0.06
18	1.00	0.76	0.69	0.52	0.33	0.21
35	1.00	0.73	0.67	0.64	0.45	0.35

*Each relative No. is calculated as the mean of 2 experiments and is represented as 1.00 of untreated control.

$$\text{Relative No. of clonogenic cells} = \frac{\text{PE (\%)} \times \text{cell No. at each time}}{\text{PE (\%)} \times \text{cell No. at 0 hr}}.$$

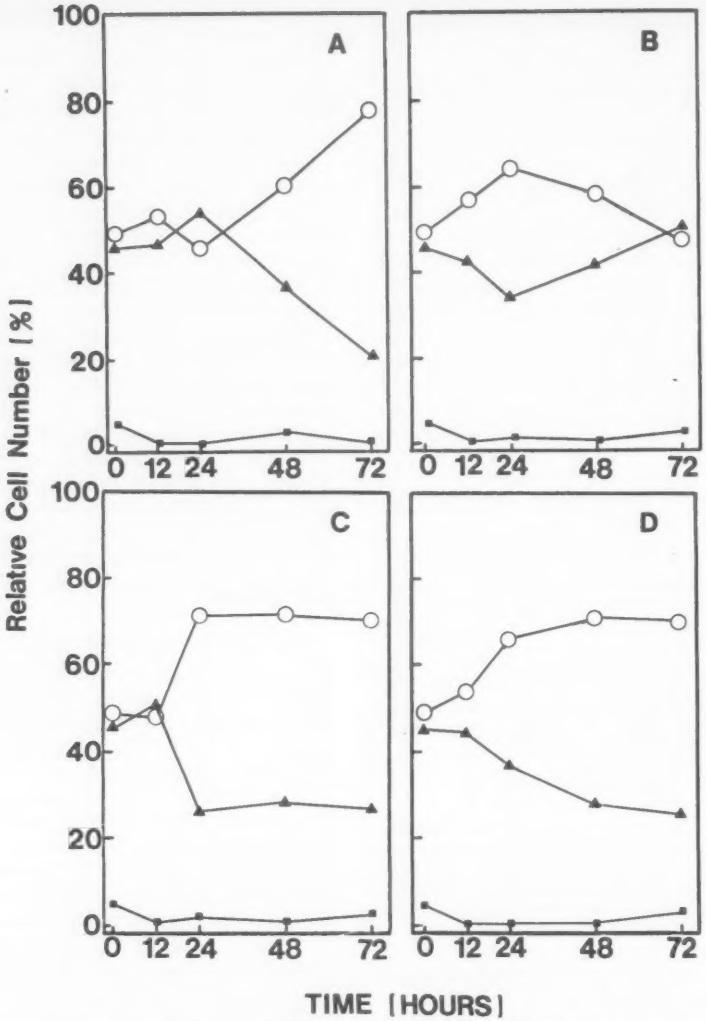

FIGURE 3.—Distribution of 9L cells in the G_1 phase (○), S phase (▲), and G_2/M phase (■) of the cell cycle after treatment with 6-MP. A, untreated; B, 2.9 μM; C, 18 μM; and D, 35 μM. Results from 2 experiments.

TABLE 2.—% of cells labeled with ^{3}H-TdR during exposure to 6-MP

6-MP concentration (μM)	% of cells labeled with ^{3}H-TdR* after—				
	0 hr	12 hrs	24 hrs	48 hrs	72 hrs
Control	41.3 ± 1.0	44.2 ± 1.9	41.2 ± 2.2	25.8 ± 3.1	16.9 ± 2.7
2.9	41.3 ± 1.0	8.3 ± 2.7	3.9 ± 0.6	3.5 ± 0.6	2.3 ± 0.2
18	41.3 ± 1.0	1.8 ± 0.3	2.4 ± 0.2	4.9 ± 1.2	3.1 ± 0.6
35	41.3 ± 1.0	2.4 ± 0.5	2.3 ± 0.1	5.4 ± 0.4	3.4 ± 0.4

*Values = mean ± SD of 3 slides; 1000 cells/slide were counted. ^{3}H-TdR: approximately 0.1 μCi/ml; specific activity of 2 mCi/mol.

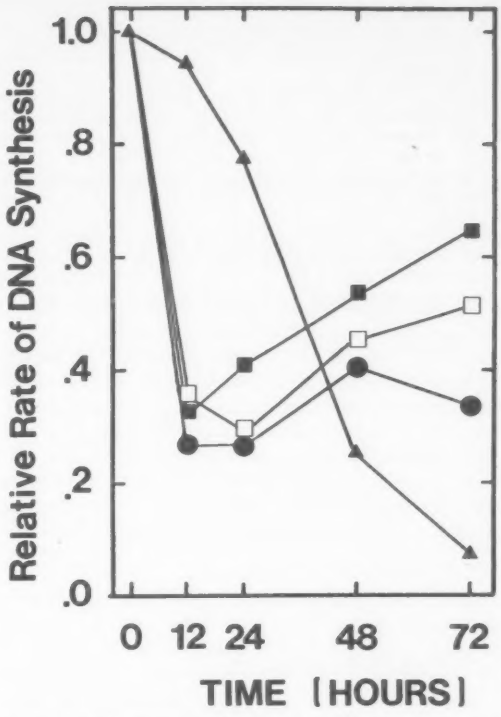

FIGURE 4.—Relative rate of DNA synthesis of 9L cells after treatment with 6-MP. ▲, untreated; ■, 2.9 μM; □, 18 μM; and ●, 35 μM. Results from 3 separate experiments.

remaining after treatment with the low dose was greater at each time point and considerably greater after total treatment times of 48 and 72 hours. However, the number of clonogenic cells that survived treatment with the low dose, normalized to the initial number of clonogenic cells, was lower than obtained by treatment with the high doses (table 1), which means that while the total number of cells was higher after treatment with the low dose, the absolute number of clonogenic cells was lower after treatment for > 48 hours.

DNA histograms showed that after treatment with low doses of 6-MP, the number of cells in G_1 phase increased for the first 24 hours and then decreased for the next 48 hours. In contrast, after treatment with the high doses of 6-MP, the number of cells in G_1 phase was high initially and remained high for 24 hours after treatment.

The results of ^{3}H-TdR labeling studies do not agree with our interpretation of the DNA histograms. While the labeling indexes (LIs) of control cells correspond approximately to the number of cells in S phase calculated from histograms, the number of cells in the treated groups, calculated from histograms, appears to be underestimated by the LI method. Incorporation of ^{3}H-TdR into cells was severely limited by 6-MP (fig 4). One possible explanation is that cells had incorporated ^{3}H-TdR that could not be identified autoradiographically. It is also possible that 6-MP caused a considerable number of treated cells to stop DNA synthesis in the middle of S phase. With our data we cannot distinguish between these possibilities.

The rapid decline in the relative rate of DNA synthesis of cells during the first 12 hours after treatment with 6-MP might be consistent with the 30%–40% cell kill. Additional cell kill would not be expected until unaffected cells cycled into S phase. The slower increase of the relative rate of DNA synthesis up to 72 hours after treatment in cells treated with high doses of 6-MP may indicate that fewer cells enter into S phase from G_1 phase at slower rates of progression. The rate of cell progression from G_1 into S phase apparently depends on the dose of 6-MP, although reduced cell kill is not strictly reflected by either the LIs or the relative rates of DNA synthesis. Therefore, treating 9L cells with 6-MP for short periods of time does not produce much cell kill even at high doses (fig 2).

Wheeler et al (14) have shown that 6-MP slows the progression to mitosis of H.Ep. 2 cells initially in S phase, does not prevent the labeling of cells initially in S phase, but does prevent or retard the progression of cells from G_1 to S phase. A similar drug-induced block at the G_1/S border, which can affect cytotoxicity, has been found for several other S phase-specific agents, but none protects cells from the cytotoxic action of drug for > 12 hours of continuous exposure (16,17).

If the concentration-dependent, self-limiting activity of 6-MP affects other cell lines and human tumor cells in the same manner that it affects 9L cells, then dose levels and the length of treatment with 6-MP in the clinical setting must be chosen carefully, and the use of high-dose 6-MP must be assessed critically.

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Attenuation of Dermal Toxicity of Doxorubicin by Liposome Encapsulation[1,2]

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The severe tissue damage which occurs when doxorubicin (Dxn) is extravasated during infusion has been attenuated by encapsulating the drug in anionic liposomes. Mice were injected intradermally with either 0.05 or 0.10 mg of Dxn in the free or liposome-entrapped form. At both dose levels, the animals receiving free drug developed dermal lesions at a higher frequency and of a greater severity than did those animals receiving Dxn-liposomes. Determination of tissue-associated fluorescence indicated that free Dxn was removed from the area of the dermal injection more rapidly than was the liposome-entrapped drug. The data suggest that the dermal toxicity of Dxn may be determined more by its mode of disposition than by the absolute amount of drug in tissue. Similar observation was made earlier for the Dxn-induced chronic cardiotoxicity. [Cancer Treat Rep 67:481–484, 1983]

Although the therapeutic usefulness of doxorubicin (Dxn) as an anticancer agent has been well-documented (1–3), serious toxic effects remain a major clinical concern. In addition to the established cardiac toxicity (4–6) and myelosuppression (7,8), Dxn can also produce significant dermatologic toxicity if extravasated during infusion. Substantial pain is noted immediately upon extravasation followed in a few hours by swelling and reddening. Skin lesions in patients can become progressively severe over several months and the full thickness of the skin can be destroyed, resulting in necrosis of the underlying tissues (9–11). In addition to deep ulcerations, there can be significant damage to nerves, muscles, and tendons. Spontaneous healing of such ulcers is rare; thus, surgical excision of involved tissues has become the recommended treatment (9,10). When affected patients are in the late stages of malignancy such aggressive treatment may be unwarranted. However, since some current therapeutic procedures now involve the use of Dxn as initial chemotherapy, the prevention and management of its extravasation have become important goals (11).

Earlier animal studies have demonstrated that the liposome encapsulation of Dxn can produce an improvement in therapeutic effectiveness of the drug (12–14). Compared to free drug, liposome-entrapped Dxn has less immune suppressive activity and a lower incidence of cardiac toxicity while retaining full antitumor potential. During the course of these animal experiments, the severe tissue damage resulting from occasional improper iv injection of free drug was clearly evident. In contrast, however, no serious tissue injury was noted following injection of Dxn-liposomes. This suggested that liposome entrapment of the drug could serve to protect tissues from its damaging effects. In this report we describe the improved therapeutic benefits of liposome encapsulation with respect to reducing skin damage produced by extravasated Dxn.

MATERIALS AND METHODS

Liposome-entrapped Dxn was prepared by methods previously described (12). Dxn was first complexed to phosphatidylcholine in a 1:2 molar ratio to avoid the formation of water-insoluble aggregates with phosphatidylserine. The resulting complex was then entrapped within anionic liposomes consisting of phosphatidylcholine, phosphatidylserine, and cholesterol in a molar ratio of 0.6:0.2:0.3/mol of Dxn. The efficiency of entrapment ranged from 5% to 10%. Following the separation of

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nonentrapped from entrapped Dxn by Sephadex G-50 chromatography, Dxn-liposomes were concentrated to 1.0 mg/ml by ultrafiltration on an Amicon P-10 filter. The recovery of intact liposomes was ≥ 98% after ultrafiltration.

Swiss Webster mice (Charles River Laboratories, Washington, DC) weighing approximately 25 g each were used in all studies. Each animal was shaved on both the right and left flanks with electric clippers 24 hours prior to treatment. Test doses consisted of either 0.05 or 0.10 ml of 1.0 mg/ml of Dxn as either free or entrapped drug. Following the procedure of Dorr et al (21), intradermal injections were made by pulling the skin tight and inserting the needle within the plane of the dermal tissue.

Skin lesions were evaluated at 1 week following the intradermal injections. Scoring was based on the severity of tissue damage: 0 = no lesion; 1 = minor induration (< 4 mm^2); 2 = moderate induration (> 4 mm^2); 3 = severe induration with erythema (> 4 mm^2); and 4 = ulceration.

The clearance of Dxn from intradermal injection sites was studied by administering 100 μg of Dxn (0.10 ml at 1 mg/ml) in either the free or liposome-entrapped form to test animals. Mice were killed at 24 and 72 hours after injection. Skin samples (1 × 2 cm) were removed and assayed for Dxn-associated fluorescence according to methods previously described (15). Tissues were homogenized in siliconized glass centrifuge tubes in a 75% ethanol-0.45 N HCl solution. After centrifugation at 40,000 × *g* for 20 minutes, supernatants were assayed for fluorescence in an Aminco-Bowman spectrophotofluorometer using excitation and emission wavelengths of 474 and 585 nm, respectively. The tissue extracts were compared to a series of Dxn standards along with

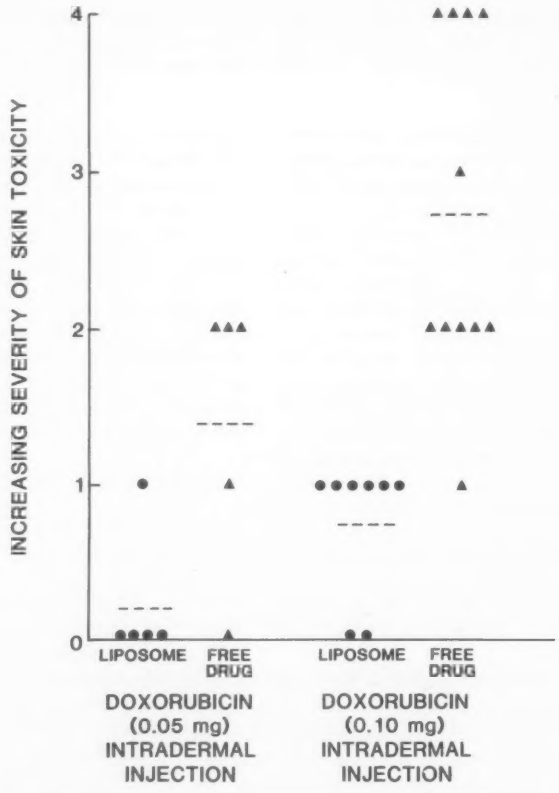

FIGURE 1.—Evaluation of Dxn-induced dermal toxicity. In 2 separate studies, mice received Dxn at doses of either 0.05 or 0.10 mg. Intradermal injections of free or liposome-entrapped drug were administered to the right and left flanks, respectively. Toxic lesions to skin were evaluated 1 wk later. Dashed lines indicate the mean toxicity scores for each test group. The protective effect of liposome encapsulation on lowering dermal toxicity was significant at both drug doses ($P < 0.001$).

control extracts from untreated mice. This technique measured total anthracycline-associated fluorescence and did not distinguish between the parent drug and its metabolites.

RESULTS

Results from the skin toxicity experiments are shown in figure 1. Four of five mice receiving 0.05 ml of free Dxn (1.0 mg/ml intradermally) displayed skin damage which ranged from minimal changes to moderate indurations with erythema. In contrast, only one of the five animals receiving an equal dose of the liposome-entrapped drug developed any sign of dermatologic toxicity, which was limited to mild induration. A larger Dxn dose of 0.10 ml (1.0 mg/ml) produced measurable lesions in all 11 mice receiving the free drug; four of the animals developed ulcerated lesions and six had moderate to severe skin damage. Equal doses of Dxn-liposomes, however, produced only mild indurations in six animals and no apparent toxicity in the remaining two. The mean toxicity scores at both dose levels were 0.54 ± 0.14 for liposome-entrapped Dxn and 2.31 ± 0.30 for free drug. This difference in toxicity scores was highly significant ($P < 0.001$).

The results from measurements of Dxn-associated fluorescence in skin samples are displayed in figure 2. These data demonstrate that the liposome-entrapped compound is more slowly removed from the injection site than is free drug. At both 24 and 72 hours, the amount of fluorescent material found in skin was significantly greater with Dxn-liposomes than for free drug ($P < 0.01$). This suggests that the Dxn-liposome preparation may be relatively stable and that the drug is less readily available for clearance from the injection site.

DISCUSSION

Extravasation of Dxn with subsequent skin necrosis continues to be a serious clinical problem despite the care with which the drug is infused (16). Current therapeutic management of dermal toxicity relies on the surgical removal of involved tissues, allowing proper healing to occur (10,17). Alternatives to surgical treatment under current investigation include the use of antioxidants (18), antihistaminic agents (18,19), nonsteroidal anti-inflammatory agents (19), corticosteroids (20,21), hyaluronidase (18,20), and β-adrenergic compounds (22). Of these, the β-antagonist propranolol

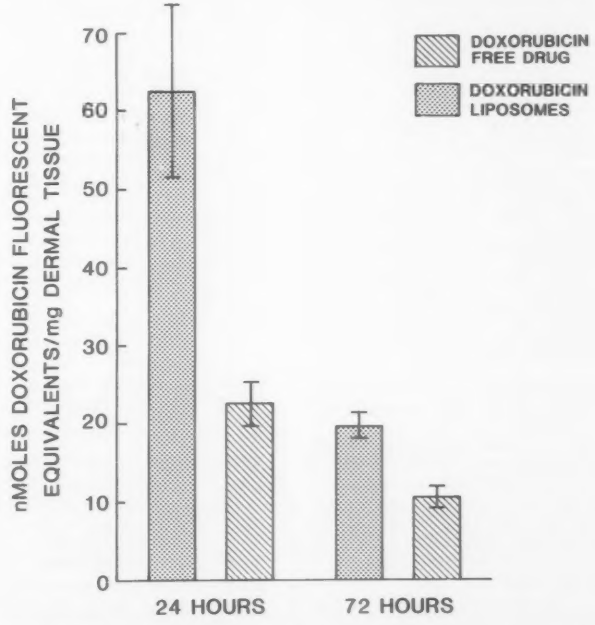

FIGURE 2.—Concentration of Dxn fluorescent equivalents in skin tissue. Dermal samples were assayed at 24 and 72 hrs following the intradermal injection of 100 μg/0.1 ml of Dxn, either free or entrapped in anionic liposomes. The association of Dxn fluorescent equivalents with dermal tissue was significantly lower for the free drug than for the liposome-entrapped material ($P < 0.01$). Values = mean ± SE (n = 5).

and the β-agonist isoproterenol have recently been shown to have some useful antidotal action, while corticosteroids have demonstrated only a limited protective effect against low doses of extravasated Dxn (21).

The entrapment of Dxn in phospholipid vesicles appears to provide significant protection against the vesicant activity normally associated with extravasation. The mechanism for this effect is not clear but may involve a sequestration of drug within liposomes at the injection site. The slower rate of clearance indicates that the entrapped drug is not as readily removed from the site of injection. Thus, Dxn may remain associated with the phospholipid vesicles and be unable to cause tissue damage. Our previous studies concerning the antitumor activity and reduced cardiac toxicity of Dxn-liposomes indicated that the observed biologic response was not proportional to the amount of drug associated with the specific tissue (23). One of the possible explanations for these observations could be that phospholipid encapsulation alters the site of action, subcellular distribution, and/or metabolism of Dxn. Previous investigators have shown that the interaction of Dxn with various phospholipids can dramatically alter its effects on membranes, which depends on the specific phospholipids present (24–26).

The reduced dermal toxicity described in this study introduces a new therapeutic benefit derived from the encapsulation of Dxn. Data from previous experiments have demonstrated other toxic effects which can be prevented or reduced by liposome entrapment. For example, young animals treated with entrapped Dxn do not display the suppression of normal weight gain as observed for equal doses of free drug (27). Encapsulation has also been shown to prevent or reduce the cardiotoxic and immune suppressive effects of Dxn while maintaining full or even improved antitumor activity (12,13). These results suggest that further studies which may lead to eventual clinical investigations are warranted to establish the potential for lessening the toxic effects of Dxn by liposome entrapment.

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Combination of Vindesine and Prednisone in Malignant Lymphoma and Acute Leukemia[1,2,3]

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Nineteen patients with advanced refractory lymphoma and 12 patients with acute leukemia, including seven in blastic crisis of chronic myelogenous leukemia (CML), were treated with vindesine in combination with prednisone. Of 16 evaluable patients with lymphocytic or histiocytic lymphoma, one achieved complete remission (6%) and eight achieved partial remissions (50%). Median duration of response was 12 weeks (range, 4–72+). Four of six evaluable patients in blastic crisis of CML showed definite improvement in blood cell counts and symptoms. The major dose-limiting toxic effect was bone marrow suppression, while neurotoxicity was seldom cause for dose modification. The study shows vindesine and prednisone to be active in heavily pretreated patients with non-Hodgkin's lymphoma and blastic phase of CML. [Cancer Treat Rep 67:485–487, 1983]

Vindesine (VDS) is a chemically derived structural analog of the vinca alkaloid, vinblastine. Its antineoplastic activity against animal tumors resembles that of another vinca alkaloid, vincristine, although it has less neurotoxicity (1–3). Prior phase II trials with VDS have been conducted in patients with acute leukemia (4), lymphoma (including Hodgkin's disease) (4,5), and solid tumors. The present study was designed to examine the usefulness of VDS in combination with prednisone in previously treated patients with lymphocytic and histiocytic lymphomas and acute leukemia in adults, especially in blastic crisis of chronic myelogenous leukemia (CML). In the acute leukemia patient group, two schedules of VDS administration were evaluated.

MATERIALS AND METHODS

The characteristics of the patient population including type of prior treatment are shown in table 1.

VDS (supplied by Eli Lilly and Co) was administered by rapid injection through an established free-flowing iv line. Lymphoma patients received VDS at a dose of 3 mg/m^2 iv weekly along with prednisone at a dose of 40 mg/m^2 orally every other day. Patients with acute leukemia were randomized to a weekly regimen of VDS at a dose of 3 mg/m^2 plus alternate-day prednisone versus VDS at a dose of 1 mg/m^2 iv daily for 5 days and prednisone at a dose of 60 mg/m^2 daily for 5 days, both repeated every 10 days. Once remission was attained in lymphoma patients, maintenance VDS was continued at 2-week intervals. VDS dosage was attenuated at evidence of marrow suppression and moderate neuropathy. Treatment was continued until evidence of progressive disease appeared.

An adequate trial of therapy consisted of at least two weekly doses or one 5-day cycle of VDS. In lymphoma patients, complete remission (CR) was defined as the complete disappearance of all disease for at least 1 month, and partial remission (PR) was defined as a $> 50\%$ reduction in measurable lesions. Lymphoma histology was classified according to the Rappaport classification (6) and acute leukemias by the French-American-British classification (7). Identification of leukemic cell type included cytochemistry but not terminal transferase.

RESULTS

Among 11 patients with stage III or IV poorly differentiated lymphocytic lymphoma, four achieved PRs of 4, 8, 12, and 16 weeks' duration and one achieved a

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TABLE 1.—Patient characteristics

Characteristic	Lymphoma patients	Leukemia patients
No. of patients evaluable	18	11
Median age in yrs (range)	65 (27–79)	61 (17–72)
Sex (male/female)	8/10	6/5
Performance status*		
0	3	0
1	7	4
2	7	4
3	0	1
4	1	2
Prior radiation therapy	4	2
Prior chemotherapy (any)	18	11
Vincristine	16	8
Vinblastine	3	0
Prednisone	16	9
Histology†	11 PDLL 5 DHL 2 HD	4 AML and AMML 1 ALL 6 BC-CML
Stage		
III	6	
VI	12	

*Performance status: 0 = asymptomatic; 1 = some symptoms, ambulatory; 2 = in bed < 50% of time; 3 = in bed > 50% of time; 4 = 100% bedridden.

†PDLL = poorly differentiated lymphocytic lymphoma; DHL = diffuse histiocytic lymphoma; HD = Hodgkin's disease; BC-CML = blastic crisis of CML.

CR of 26 weeks' duration, preceded by a PR of 13 weeks' duration. In five patients with histiocytic lymphoma there were no CRs, but four PRs lasting 6, 10, 18, and 72+ weeks were seen. Of the two patients with Hodgkin's disease, one had a PR of 13 weeks, while the other showed no response.

Of the five adult patients with acute leukemia (one patient with acute lymphocytic leukemia [ALL] and four with acute myelocytic [AML] or acute myelomonocytic [AMML] leukemia), only one patient with AML showed brief peripheral blood improvement lasting 4 weeks. This patient and three of the other four patients had previously received vincristine and prednisone. Six patients were treated during blastic phase of CML. All of these patients had myelocytic or myelomonocytic blast cells. Four patients showed dramatic, though brief, reduction in their wbc counts (196,000 to 15,500/mm^3, 115,000 to 2200/mm^3, 157,000 to 600/mm^3, and 12,500 to 3000/mm^3) lasting 4, 4, 8, and 9 weeks. Improvement in blood cell counts was associated with definite symptomatic benefit, including temporary clearing of retinal infiltrates in one patient. Two of the four responders had received vincristine and prednisone prior to VDS, but had not responded. Responders were divided equally between the two treatment arms. It was of interest that all four responders had chromosomal abnormalities (two with Ph′, one with trisomy 8, and one with absent C- chromosome), while the two nonresponders had no major chromosomal abnormalities.

Hematologic toxicity in our lymphoma patients was manifested by an average drop of 1.9 g/100 ml in hemoglobin during the treatment period (range, 0–4.0 g/100 ml). Mild to moderate wbc count suppression was seen in eight of 18 patients (44%), and severe suppression (< 2000/mm^3) in four (22%). Platelet counts were suppressed to < 100,000/mm^3 in only one patient, and actually rose to > 500,000/mm^3 in two patients during VDS treatment.

The degree of neurotoxicity observed in both our lymphoma and leukemia patients was generally mild and readily reversible. Mild to moderate sensory neuropathy was reported in 17 of 25 patients (59%) and severe changes were reported in one (3%). Mild to moderate motor weakness was seen in six patients (21%) and mild constipation was observed in one (3%). In the leukemia patients, the intermittent daily doses of VDS resulted in similar neuropathy as did the weekly regimen.

DISCUSSION

We have demonstrated VDS combined with prednisone to be an effective chemotherapeutic agent in heavily pretreated patients with lymphocytic and histiocytic lymphoma and in blastic phase of CML. In the non-

Hodgkin's lymphoma group one patient (6%) achieved CR and eight (50%) achieved PR, with a median duration of response of 12 weeks (range, 4–72+). In a study by Sklaroff et al (5), one CR lasting 12+ months was observed in ten non-Hodgkin's lymphoma patients and four PRs were observed in 20 Hodgkin's disease patients. Mathé et al (4) reported two PRs among 13 patients with lymphosarcoma and two PRs among five patients with Hodgkin's disease. One of two patients with lymphoma included in the series of patients reported by Valdivieso et al (8) achieved a PR.

In our six patients in blastic crisis of CML, four responses were observed; these occurred in patients with a nonlymphocytic blast cell population. Although these responses resulted in marked improvement of peripheral blood blast numbers and symptoms, these remissions could not be maintained for long. Median duration of remission was 6 weeks (range, 4–7). Mathé and coworkers (4) also investigated VDS as a single agent in previously treated leukemia patients. In 15 patients with ALL, six achieved CR and one achieved PR. In three AML patients, no responses were seen, but in six patients in blastic phase of CML, three CRs were observed. These results obtained in blastic phase of CML are encouraging if, by the addition of other active agents, the initial good responses can be maintained for longer periods of time. Recent studies in mice inoculated with L1210 leukemia suggest a schedule-dependent synergism of VDS when given in combination with methotrexate (9). By exploring this and other combination programs more prolonged remissions may possibly be achieved with VDS.

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Phase II Study of Amsacrine (*m*-AMSA) in Advanced Lymphomas: A Southwest Oncology Group Study[1,2]

James K. Weick,[3] Stephen E. Jones,[4] and Donna H. Ryan[5,*]

Amsacrine was administered to 73 evaluable patients with previously treated malignant lymphoma in the Southwest Oncology Group. The drug was administered iv every 3 weeks at a dose of either 90 mg/m² to poor-risk patients (38 patients) or 120 mg/m² to good-risk patients (35 patients) in 500 ml of 5% dextrose in water over 1 hour. The overall complete plus partial response rate is 16% (12 responses among 73 evaluable patients), with a response duration of 1–33 months. The dose-limiting toxic effect was myelosuppression, with leukopenia in 29 patients and thrombocytopenia in 18 patients. Our experience in the Southwest Oncology Group demonstrates the activity of amsacrine in malignant lymphoma. [Cancer Treat Rep 67:489–492, 1983]

Since the introduction in 1933 of acridine dyes which possessed tumor activity, numerous modifications of the acridine nucleus have been made attempting to increase the therapeutic index. The current drug, amsacrine (*m*-AMSA), is one of the many active derivatives which were prepared by Cain and Atwell (1). In phase I and early phase II studies, *m*-AMSA exhibited a wide spectrum of activity in breast cancer, melanoma, lymphoma, ovarian cancer, and acute leukemia (2,3).

The mechanism of action of *m*-AMSA has not been entirely clarified. The compound does affect DNA synthesis in L1210 cells in the mouse, and in in vitro systems it removes and reverses the coiling of DNA, consistent with the hypothesis that *m*-AMSA binds to DNA through intercalation (4,5). Other mechanisms of action on cellular DNA have also been considered (6). The Southwest Oncology Group (SWOG) evaluated this compound in malignant lymphomas (both Hodgkin's disease [HD] and non-Hodgkin's lymphoma [NHL]) between July 1979 and April 1981.

PATIENTS AND METHODS

Seventy-seven patients, 17–78 years of age (median, 44.8 years), were registered in this SWOG study No. 7918. Seventy-three patients were treated and were evaluable for response. Two patients were not eligible on pathologic review because no lymphoma was present on the tissue provided and two patients elected not to receive treatment after registration and are not considered in the evaluation. Histologic diagnoses and response data are shown in table 1. Patients with either HD or NHL were eligible for this study with no age restrictions. An average of 2.6 different chemotherapy combinations had been administered to the group of patients and 37 patients had also received radiation therapy. Ten patients had not responded to prior treatment programs; the quality and duration of response to prior therapy were not recorded in the remaining patients, only that there had been a response. Other requirements for inclusion in the study included measurable disease, a wbc count $> 3500/mm^3$, and a platelet count $> 100,000/mm^3$, unless lower counts were due to marrow involvement by lymphoma. Adequate renal function was required as manifested by a BUN of < 20 mg/100 ml and a serum creatinine of < 1.2 mg/100 ml. Patients were required to have an adequate liver function as defined by a bilirubin of < 2 mg/100 ml, with no clinical or chemical evidence of biliary obstruction. All patients gave written informed consent in accordance with institutional policies before therapy was administered.

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TABLE 1.—Characteristics of 73 patients receiving *m*-AMSA

Histologic type	No. of patients	Response	
		No.	Duration (mos)
HD			
Nodular sclerosis	14	2	2,3
Mixed cellularity	4	1	8
Lymphocyte predominant	1	0	—
Lymphocyte depleted	1	1	5
Total	20		
NHL, diffuse			
Histiocytic	17	1 (complete)	9
Poorly differentiated	13	2	12,33
Well-differentiated	4	2	1,4
Mixed	2	0	—
Undifferentiated	2	1	3
Lymphoblastic	2	0	—
Total	40		
NHL, nodular			
Poorly differentiated	9	2	1,4
Mixed	2	0	—
Total	11		
Other			
Cutaneous T cell	2	0	—
Total	2		

*m*AMSA was given at a dose of 90 mg/m^2 to poor-risk patients defined as having one or more of the following: > 65 years old, prior whole abdominal or pelvic radiation, severe myelosuppression with previous chemotherapy, and initial wbc counts of < 3500/mm^3 and/or platelet counts of < 100,000/mm^3, as well as a bilirubin > 1.2 but < 3 mg/100 ml. A dose of 120 mg/m^2 was established for those good-risk patients < 65 years old, with no prior abdominal or pelvic radiation and with normal liver function. Pretreatment performance status was rated as SWOG 0 or 1 (Karnofsky, 70–100) in 19 patients and 2–4 (Karnofsky, 10–60) in 54 patients. The drug was administered iv in 500 ml of 5% dextrose in water over 1 hour and was repeated at 3–4-week intervals if pretreatment counts had been achieved. Thirty-five patients were treated on the good-risk arm and 38 patients were treated on the poor-risk arm. When it became apparent that severe cardiac toxicity was a possible side effect of *m*-AMSA, especially with reports of dysrhythmias, ventricular fibrillation, and cardiac arrest, electrocardiograms and serum potassium were obtained before each infusion. A complete remission (CR) was defined as the complete disappearance of all evidence of disease and symptoms related to the disease for a minimum of 1 month. An adequate trial was considered to be two courses of therapy. A partial remission (PR) was defined as a ≥ 50% reduction in the sum of the product of the diameters of measured lesions, lasting for at least 1 month. No simultaneous increase in the size of any lesion or the appearance of new lesions could occur. Patients were removed from the study if there was increasing disease after two courses of therapy and/or if unacceptable toxicity developed.

RESULTS

Of the 75 eligible patients, 66 are fully evaluable for response and seven are partially evaluable. In the latter group, these seven patients received only one course of treatment and died between Days 1 and 15; they are considered to be early deaths. In each instance, the investigator determined that the cause of death was progressive disease rather than drug toxicity. Thus, 73 patients are fully or partially evaluable and are the basis of this report.

Responses are shown in table 1. The overall CR plus PR rate is 16% (12 responses among 73 evaluable patients). If early deaths are excluded, this rate becomes 23% (12 responses among 66 patients). The durations of response varied from 1 to 33 months in the 11 partial responders and 9 months in the single complete responder. Except for the single 33-month responder, most responses were short and varied from 1 to 12 months. The patient with the longest remission had disease seen only in the bone marrow at the time of entrance in the study and was removed from all therapy after 12 courses of *m*-AMSA were administered. At the time of last follow-up, the bone marrow aspirate and biopsy are nor-

TABLE 2.—Toxicity of *m*-AMSA

Toxicity	Degree				
	Mild	Moderate	Severe	Life-threatening	Total
Leukopenia	4	11	8	6	29
Thrombocytopenia	5	4	5	4	18
Nausea and vomiting	10	7	2	0	19
Cardiac toxic effects	1	0	0	0	1
Depression	1	0	0	0	1

mal and peripheral values have returned to normal. By disease category the response rates for evaluable patients for HD, NHL (nodular), and NHL (diffuse) are 20% (four of 20 patients), 18% (two of 11), and 15% (six of 40), respectively. The two patients listed as "other" are patients with cutaneous T-cell lymphoma in whom no response was seen. All responders had received prior doxorubicin therapy. Of the 12 responders, two had failed prior chemotherapy programs and another patient had failed both prior chemotherapy and radiation therapy; the response durations in those three patients were 3, 4, and 4 months, respectively.

One hundred and ninety-three courses were administered to the 73 patients for an average of 2.6 courses per patient, and the toxicity of these courses is detailed in table 2. Table 3 defines the criteria used to determine toxicity in this study. This toxicity is consistent with earlier reports, including mainly myelosuppression which was significant in a number of patients. Leukopenia occurred in a total of 29 patients and was severe or life-threatening in 14 (19%). Thrombocytopenia was documented in a total of 18 patients and was severe or life-threatening in nine (15%). An equal number of those patients with severe or life-threatening hematologic toxic effects were in the good-risk and poor-risk categories. In addition, there was no correlation with the presence or absence of bone marrow involvement or prior duration of chemotherapy and radiation therapy.

Gastrointestinal toxicity was minimal and consisted of nausea, vomiting, mucositis, and diarrhea. Except for two instances of sinus tachycardia observed before the third course of chemotherapy, the only cardiac toxic effect seen in the study was in one patient with a right bundle branch block and first-degree atrioventricular block, which may not have been related to drug administration.

Except for the previously mentioned responder with bone marrow infiltration, the remaining responses were in patients with primarily nodal disease. No other responses were seen in patients with extranodal disease.

DISCUSSION

The results of this study indicate that *m*-AMSA has tolerable toxicity and is beneficial in 16% of patients with advanced resistant lymphoma when given as a single dose every 21 days. The dose-limiting toxicity was myelotoxicity. In a similar report, Warrell et al (7) in a smaller group of patients reported no responders in 15 patients with NHL. Thus, considering the heavy

TABLE 3.—Chemotherapy toxicity criteria

Toxicity	Degree				
	None	Mild	Moderate	Severe	Life-threatening
Wbc count (granulocytes/μl)	> 4000 (> 1500)	3000–3999 (< 1500)	2000–2999 (< 1000)	1000–1999 (< 500)	< 1000 (< 250)
Platelet count (cells/μl)	≥ 100,000	75,000–99,999	50,000–74,999	25,000–49,999	< 25,000
Nausea and vomiting	Normal	Nausea, no vomiting	Vomiting can be reduced by treatment to < 6 × day	Vomiting > 6 × day despite antiemetics	
Cardiac toxic effects	Normal in all	ECG changes only, or sinus tachycardia not to exceed 110 at rest	Arrhythmias, except ventricular tachycardia ± ECG changes	Congestive heart failure, ventricular tachycardia, pericarditis, and/or effusion	

pretreatment in this patient population, we conclude that *m*-AMSA is of therapeutic utility in advanced NHL and HD, and merits serious consideration for incorporation into new combination chemotherapy programs.

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Phase II Trial of α(Human Leukocyte) Interferon Administered Daily in Adenocarcinoma of the Colon/Rectum[1]

Robert A. Figlin,* Mary Callaghan, and Gregory Sarna[2,3]

Nineteen patients with metastatic adenocarcinoma of the colon or rectum were treated with α(human leukocyte) interferon (αIFN). Patients received 3 × 10^6 IU/day of interferon im 5 days/week. Eighteen patients are evaluable for response and toxicity. There were no objective responses. All evaluable patients demonstrated progression of disease during therapy. Toxicity consisted primarily of mild granulocytopenia, thrombocytopenia, fatigue, weakness, and fever. This trial demonstrates that while αIFN can be administered safely as scheduled, this regimen has no activity against adenocarcinoma of the colon/rectum.
[Cancer Treat Rep 67:493–494, 1983]

Interferons are glycoproteins which have both antiviral and antineoplastic activity. Clinical trials have demonstrated interferon-induced tumor regression in multiple myeloma, metastatic breast cancer, non-Hodgkin's lymphoma, and renal cell carcinoma (1–5). The exact role interferons will take in the therapy of cancer is yet to be defined. Efficacy and toxicity of the optimum interferon as a function of class (α,β,γ), source (leukocyte, lymphoblast, fibroblast, recombinant), dose schedule, and target tumor are currently undergoing intense study. Because effective chemotherapy for advanced colorectal cancer remains elusive, we have studied α(human leukocyte) interferon (αIFN) given on a 5-day/week schedule in order to assess its potential activity in patients with metastatic adenocarcinoma of the colon/rectum.

PATIENTS AND METHODS

αIFN, prepared from buffy coats, was obtained from the New York Blood Center (through the Interferon Foundation). The αIFN was prepared in 0.01 M sodium phosphate buffer (pH 7.0) and was 0.1%–1.0% pure. Three lots (119, 120, and 121) were used during this study. The specific activity ranged from 2.09 to 2.28 × 10^6 μ/mg of protein. Interferon activity was measured using the cytopathic effect inhibition assay. Sendai virus and hepatitis surface antigen were absent from lots tested.

Between October 1981 and July 1982, 19 patients with metastatic adenocarcinoma of the colon or rectum were entered into this study. Patients were required to have measurable disease. For hepatic lesions, this was defined as a liver defect ≥ 5 cm in diameter by liver-spleen scan or computed tomography, or malignant hepatomegaly extending ≥ 5 cm below the costal margin. Abdominal disease measured by computed tomography also had to be ≥ 5 cm in diameter. Bone lesions, effusions, and tumor markers (eg, CEA) were not considered measurable. On-study evaluation consisted of physical examination, baseline laboratory tests, chest x-ray, and measurement of marker areas. Patients were required to be ambulatory with leukocyte counts > 3000/mm^3 and platelet counts > 85,000/mm^3. Patients could not have received antineoplastic therapy (chemotherapy, surgery, radiation, or immunotherapy) in the preceding 4 weeks, nor previous therapy with interferon of any type.

Patients received 3 × 10^6 IU/day of αIFN im 5 days/week (Monday through Friday). This schedule was chosen to facilitate outpatient administration and allow a weekly recovery period during which the toxicity of interferon might abate. The dosage chosen is similar to that which showed activity in studies of osteogenic sarcoma, non-Hodgkin's lymphoma, breast cancer, and multiple myeloma (1–6). Patients were followed at 3-week intervals or as necessary. Patient characteristics are presented in table 1. All but one patient had re-

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[3]We thank the "Interferon Foundation" of Houston, TX, for providing the interferon for this study.
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TABLE 1.—Patient characteristics and results

Characteristic	No. of patients (%)
Total/evaluable	19/18
Median age (range): 59 (43–71)	
Male/female: 9/9	
Median performance status (range): 70 (50–100)	
Sites of measurable disease	
Liver	13 (72)
Lung	9 (50)
Pelvis and retroperitoneum	5 (28)
Prior cytostatic treatment	
None	1 (5)
1 agent	5 (28)
> 1 agent (2–5)	12 (67)
Toxicity	
Hematologic	
Nadir wbc count (cells/mm^3)	
> 4000	10 (56)
3000–4000	3 (17)
1500–3000	5 (27)
Nadir platelet count (cells/mm^3)	
> 150,000	13 (72)
100–150,000	2 (11)
50–100,000	3 (17)
Nausea (mild)	7 (39)
Fatigue/weakness	
None	1 (6)
Mild	8 (44)
Moderate	6 (33)
Severe	3 (17)
Pain at injection site	13 (72)
Fever (range, 99.6°F–103°F)	18 (100)
Response	
Objective	None
Disease progression	18 (100)
Median time to disease progression (range): 5.5 wks (1–12)	

ceived previous chemotherapy. The following response categories were used: complete response was defined as disappearance of all evidence of disease lasting ≥ 4 weeks; partial response was defined as ≥ 50% decrease in the sum of the product of diameters of marker lesions (or for malignant hepatomegaly the sum of the extent below the xiphoid and the costal margins at the midclavicular line) lasting ≥ 4 weeks and without appearance of new lesions or growth of old lesions; minimal response was defined as objective shrinkage of disease not of sufficient magnitude to be classed as a partial response but lasting ≥ 4 weeks without appearance of new lesions or growth of old lesions. Objective response was defined as complete response, partial response, or minimal response. Progression was defined as the appearance of new lesions, tumor-induced death, or an increase of > 25% in measured tumor areas. All other responses were classified as stable. Patients receiving greater than or equal to five doses of αIFN were considered evaluable.

RESULTS

Eighteen of the 19 patients were evaluable for response and toxic reactions (table 1). The single nonevaluable patient was found to be thrombocytopenic from an independent cause after receiving only one injection of αIFN. No objective responses were observed. No patient had stabilization of disease. All 18 evaluable patients demonstrated progression of disease during therapy, with a median time to progression of 5½ weeks. Toxicity for this dose schedule was tolerable and compatible with previous reports (1–5). It consisted primarily of mild granulocytopenia, thrombocytopenia, fatigue, weakness and fever, and is also detailed in table 1. Constitutional symptoms tended to abate in the 2 days/week in which interferon was not given. Hepatic toxicity, hypotension, neurotoxicity, herpetic eruptions, and alopecia were not observed.

DISCUSSION

In this phase II study of interferon as therapy for advanced colorectal cancer we have detected no activity of αIFN administered according to the chosen schedule. With no objective responses in 18 consecutive patients, $P = 0.982$ that the true response rate is < 20% and $P = 0.95$ that the true response rate is < 15%. The median time to progression is not different from that of a comparable group of 44 patients we have studied with metastatic adenocarcinoma of the colon/rectum treated with but not responding to laetrile (C. Moertel, personal communication). The toxicity of αIFN in this schedule was significant but acceptable. In conclusion, while αIFN can be administered safely as scheduled, this chosen regimen demonstrates no activity against previously treated patients with adenocarcinoma of the colon/rectum.

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Lack of Inhibition by Oxipurinol of 5-FU Toxicity Against Human Tumor Cell Lines[1,2]

Harinder S. Garewal,[3] Frederick R. Ahmann,[4] and David S. Alberts[3,*]

Allopurinol has been reported to decrease the gastrointestinal and bone marrow toxicity of 5-FU when administered in a high dose by continuous infusion. The effect of oxipurinol, the major metabolite of allopurinol, on 5-FU cytotoxicity against three human tumor cell lines was studied using a soft agar clonogenic assay. For both WiDR (colon) and T-47 (breast), 5-FU cytotoxicity was greater in the presence of oxipurinol than in its absence. Oxipurinol did not significantly affect 5-FU cytotoxicity against Hec-1A (endometrial). In none of the three lines was a protective effect of oxipurinol noted. [Cancer Treat Rep 67:495–498, 1983]

Allopurinol, a drug commonly used to treat hyperuricemia, is also known to protect normal gastrointestinal and bone marrow tissue from 5-FU toxicity in rodents and humans (1–3). This effect is mediated through oxipurinol which is the major metabolite of allopurinol (3). Oxipurinol is phosphorylated by hypoxanthine-guanine phosphoribosyltransferase to 1-oxipurinol-5′-monophosphate which is a potent inhibitor of the enzyme orotidylate decarboxylase (ODCase) (4–6). This inhibition of ODCase leads to accumulation of orotic acid which competes with 5-FU for orotate phosphoribosyltransferase, a key enzyme involved in the metabolism of 5-FU to ribonucleotides (4,5). Oxipurinol is also activated to 7-oxipurinol-5′-monophosphate via a pyrimidine phosphoribosyltransferase which is an inhibitor of OMP decarboxylase.

5-FU must be converted intracellularly to a nucleotide derivative in order to produce a cytotoxic effect by interfering with nucleic acid synthesis (7,8). The first step is the formation of fluorouridine monophosphate which can occur by at least two different pathways: (*a*) direct conversion of 5-FU to fluorouridine monophosphate via orotate phosphoribosyltransferase, or (*b*) sequential action of uridine phosphorylase and uridine kinase using ribose-1-phosphate. Therefore, a theoretic basis exists whereby different tissues may demonstrate different degrees of modulation of 5-FU cytotoxicity by allopurinol based on whether or not they depend primarily on the orotate phosphoribosyltransferase pathway for 5-FU activation.

In cancer treatment, the clinical utility of the protective effect of allopurinol on normal tissue will clearly depend on whether or not a similar protection exists in malignant tissues. Previous in vitro investigations of the effect of allopurinol on 5-FU cytotoxicity have used cell lines in liquid culture (9). However, no studies have been performed with human tumor cell lines in semisolid media to analyze the interaction of oxipurinol and malignant cells.

The soft agar clonogenic assay has been shown to predict tumor resistance or sensitivity to standard cytotoxic anticancer drugs (10,11). In this paper we have used the soft agar assay to study the interaction of 5-FU and oxipurinol using three human tumor cell lines.

MATERIALS AND METHODS

Three human tumor cell lines (*see* table 1) were selected based on their sensitivity to continuous exposure

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TABLE 1.—Cell sources

Source	Cell line	Tissue	Medium*	Harvesting
American Type Culture Collection (Rockville, MD)	WiDR	Colon	RPMI 1640	Trypsin-EDTA
George Washington University (Washington, DC: Dr. O. Alabaster)	T-47	Breast	McCoy's 5A	Trypsin-EDTA
Memorial Sloan-Kettering Cancer Center (New York, NY)	Hec-1A	Endometrial	McCoy's 5A	Tyrodes

*10% fetal calf serum (Flow Laboratories); 1% penicillin (100 units/ml)-streptomycin (1 mg/ml) (Gibco).

to 5-FU in the soft agar assay. They were WiDR (colon), Hec-1A (endometrial), and T-47 (breast).

A double-layer soft agar assay was used as described by Hamburger and Salmon (12,13). Single-cell suspensions were diluted and plated out on culture dishes at final concentrations of 20,000 cells for WiDR, 40,000 cells for Hec-1A, and 50,000 cells for T-47/plate. Each experiment included triplicate studies for every 5-FU or 5-FU plus oxipurinol concentration tested. Colonies > 60 μm in diameter were counted after 7–10 days of growth using a Bausch and Lomb Omnicon FAS II automated image analyzer which had been optimized for tumor colony counts. Results were expressed as the percentage of colony formation as compared to control plates not exposed to drug.

Dose-response curves in the presence and absence of oxipurinol were statistically analyzed with a test for the equality of two regression lines using a partial F statistic (14).

RESULTS

Effect of Oxipurinol

Control experiments were done with each of the cell lines using 10^{-4} M oxipurinol alone. No significant effect of oxipurinol was observed on the amount of colony formation, the number of colonies formed in the presence of oxipurinol being within 10% of that observed in its absence with each of the three cell lines.

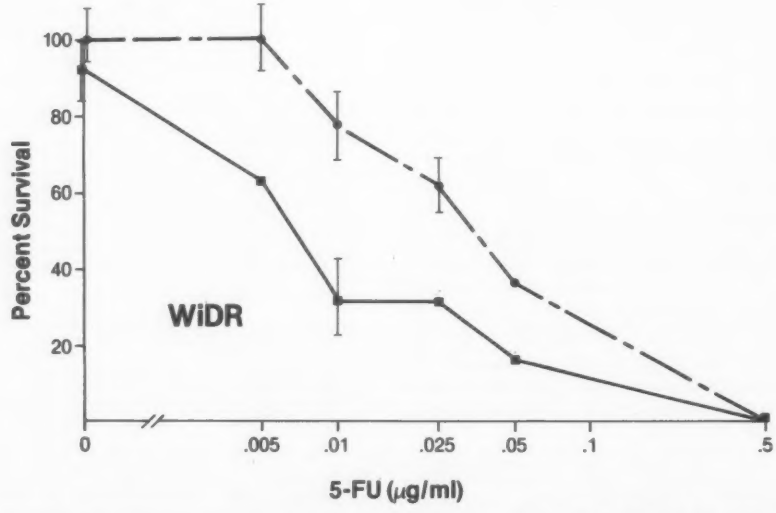

FIGURE 1.—Dose-response curves of 5-FU against WiDR (colon) in the absence (broken line) and presence (solid line) of 10^{-4} M oxipurinol. Bars are used to indicate SD based on triplicate assays shown only if it exceeded ± 5%.

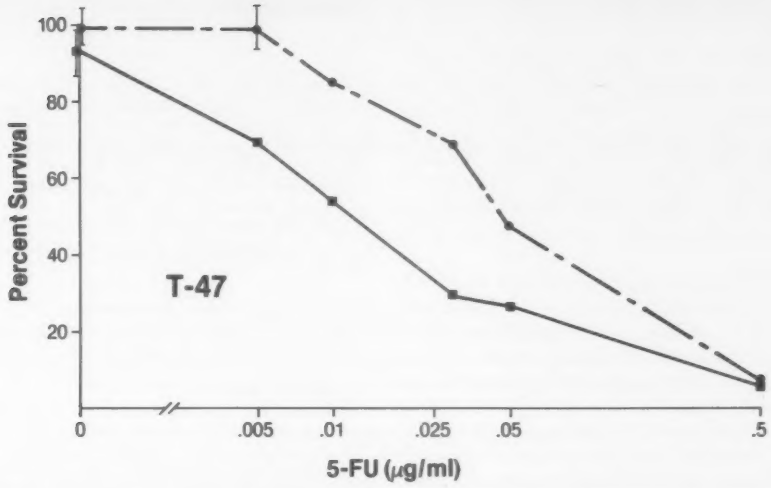

FIGURE 2.—Dose-response curves of 5-FU against T-47 (breast) in the absence (broken line) and presence (solid line) of 10^{-4} M oxipurinol. Bars are used to indicate SD based on triplicate assays shown only if it exceeded ± 5%.

Effect of Oxipurinol on 5-FU Dose-Response Curves

The dose-response curves for 5-FU (continuous exposure) for the three cell lines in the absence and presence of 10^{-4} M oxipurinol are shown in figures 1–3. For the WiDR and T-47 cell line (figs 1 and 2) there was a statistically significant difference between the curves in the presence and absence of oxipurinol ($P = 0.004$ and 0.002, respectively). For both of these cell lines, the 5-FU concentration required to produce 50% colony kill was reduced by a factor of approximately 5 in the presence of 10^{-4} M oxipurinol. As shown in figure 3, the dose-response curves for Hec-1A in the presence and absence of oxipurinol were not significantly different.

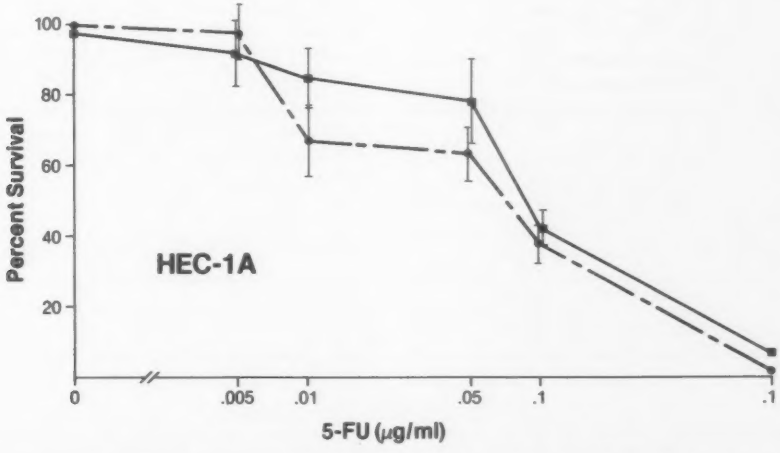

FIGURE 3.—Dose-response curves of 5-FU against Hec-1A (endometrial) in the absence (broken line) and presence (solid line) of 10^{-4} M oxipurinol. Bars are used to indicate SD based on triplicate assays shown only if it exceeded ± 5%.

DISCUSSION

We have utilized the soft agar clonogenic assay technique to study the interaction between 5-FU and oxipurinol in vitro using three different human tumor cell lines. Instead of finding a protective effect, the combination of oxipurinol and 5-FU caused greater inhibition of colony formation than did 5-FU alone in two of the three lines. In the third cell line, oxipurinol did not significantly alter the dose-response curve for 5-FU alone.

Previous in vitro experiments have used liquid culture techniques with findings of either no effect or a protective effect (9). It is probable that different metabolic pathways for 5-FU activation exist in different cell lines. This premise is supported by the results of our work as well. However, the soft agar clonogenic assay has the advantage of being able to predict tumor resistance or sensitivity to standard cytotoxic anticancer drugs when applied to tumor tissue obtained from cancer patients (10,11). It would be of interest to use the assay to study the interaction of oxipurinol and 5-FU on fresh human tumors which proved sensitive to 5-FU in vitro.

Most clinical studies with the combination of allopurinol and 5-FU have been done in colorectal cancer (1,2,15,16). Phase I studies have demonstrated that with allopurinol pretreatment a twofold higher dose of 5-FU could be given by continuous infusion leading to a fourfold higher serum concentration (2). In gastrointestinal malignancy a 50% response rate was found in an initial report (1). However, subsequent studies have failed to reproduce this for colorectal cancer (15,17). In one study a few patients with other malignancies were included and clinical responses were noted (2). The results of our tumor cell line studies suggest that the allopurinol/5-FU interaction may vary in different tumor types. More phase II studies using patients with different types of cancer are clearly needed to assess whether this combination will prove more effective than 5-FU alone in some patients.

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Failure of DMSO and Vitamin E To Prevent Doxorubicin Skin Ulceration in the Mouse[1,2]

Robert T. Dorr* and David S. Alberts[3]

Doxorubicin (DOX)-induced skin ulceration in rats and pigs has been reported to be reduced when treated with topical DMSO and/or vitamin E. In the present study using a mouse model, neither intradermal nor topical DMSO with or without vitamin E, administered up to 7 days, reduced intradermal DOX-induced skin ulceration. Intradermal DMSO with or without vitamin E caused skin ulceration and significantly increased DOX-induced ulcerations. Topical DMSO-containing solutions were not toxic to mouse skin. To test for a systemic effect of topical DMSO, two groups of mice received an additional 0.05-mg intradermal injection of DOX above a DMSO-treated lesion. There was no apparent effect of topical DMSO with or without vitamin E on this proximal but untreated DOX lesion. The results suggest either a major difference in DOX ulceration characteristics between rats and pigs on the one hand and mice on the other hand or a lack of significant efficacy for DMSO and vitamin E as DOX extravasation antidotes. [Cancer Treat Rep 67:499–501, 1983]

Inadvertent venous extravasation of doxorubicin (DOX) can result in severe tissue necrosis in man (1). A variety of pharmacologic antidotes have been suggested as being moderately effective in ameliorating the local soft tissue damage. These include low doses of glucocorticosteroids (2), beta-adrenergic agents (3,4), and adenosine (4). The free-radical scavenging agents DMSO and vitamin E (tocopheryl salts) have been reported recently to reduce in vivo DOX-induced ulceration in pig (5) and in rat (6,7) skin. Thus, DMSO has been recommended for the treatment of DOX extravasations in human skin (7). To confirm the activity of DMSO and vitamin E as antidotes for DOX-induced skin ulceration, we have used an established mouse model (8) to quantitate these localized drug interaction effects.

METHODS

Adult female BALB/c mice were used. Their hair was removed with a topical depilatory (Neet lotion) 24 hours prior to the intradermal injection of 0.05 or 0.5 mg of DOX (approximately 7 and 70 mg/m^2, respectively). Local intradermal DOX antidotes included 0.05 ml of 0.9% sodium chloride USP (control), DMSO (90% wt/vol; Burdick and Jackson, Muskegon, MI), and alpha-tocopheryl acetate (10 mg/0.05 ml; US Vitamin, Tuckahoe, NY). Antidotes were also applied topically to the DOX lesion to duplicate earlier positive trials (7); these included 0.5 ml of DMSO (90% wt/vol daily for 7 days) and 0.5 ml of a mixture of DMSO (90% wt/vol) and alpha-tocopheryl succinate (10% wt/vol; Sigma Chemical Co, St. Louis, MO). Animals receiving this combined DMSO-vitamin E regimen were given an additional intradermal injection of 0.05 mg of DOX approximately 1 cm above the initial intradermal DOX-induced lesion to test for a "systemic" absorption effect of DMSO on DOX-induced skin lesions. Only the lower DOX-induced lesion (0.05 or 0.5 mg intradermally) was treated with DMSO and vitamin E. All lesions were evaluated daily for measurement of the widest perpendicular widths of induration, erythema, and ulceration. The product of the ulceration area times day was calculated to quantitate an area under the curve (AUC) or the sum of total ulceration over time. Intergroup statistical analyses compared lesion AUC (cm^2 × days), peak size (cm^2), and duration of ulceration (days) using analysis of variance and the Student-Newman-Keuls (SNK) multiple-range test at a 0.05 level.

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TABLE 1.—Mean data for 9 antidotal regimens

Antidote	Total toxicity AUC (cm^2 × days) at DOX dose (mg)—			Peak level (cm^2), at DOX dose (mg)—			Damage duration (days) at DOX dose (mg)—		
	None	0.05	0.5	None	0.05	0.5	None	0.05	0.5
None	—	0.520	8.235	—	0.069	0.660	—	17.8	29.5
Normal saline intradermally (control)	0	0.301	6.005	0	0.069	0.560	0	8.2	27.3
DMSO (90% 0.5 ml × 7 days) topical	0	0.413	9.836	0	0.078	0.860	0	9.75	33.2
0.05 ml of DMSO intradermally	2.955*	1.064*	11.786*	0.460*	0.222*	1.111	23.5	15.8	26.8
0.05 ml of DMSO + 10% vitamin E intradermally	3.5125*	ND†	ND	0.765*	ND	ND	13.0	ND	ND
d,l-alpha-tocopheryl acetate (10 mg) intradermally	0	0.132	12.17*	0	0.028	0.816	0	5.2	24.1
DMSO (90% wt/vol) + alpha-tocopheryl succinate (10% wt/vol), 0.05 ml topically × 7 days									
Treated lesion	0	0.111	6.94	0	0.025	0.648	0	6.8	29.8
Nearby untreated lesion (induced by 0.05 mg of DOX) proximal to lesion induced by —									
0.05 mg of DOX	—	0.228	—	—	0.043	—	—	7.2	—
0.5 mg of DOX	—	0.340	—	—	0.063	—	—	8.5	—

*$P < 0.05$ for group, compared to subset containing controls (saline or DOX alone) using SNK multiple-range test.
†ND = experiment not done.

RESULTS

Table 1 shows the mean data for the nine antidotal regimens, each tested against the two intradermal DOX doses of 0.05 and 0.5 mg. Painting a 90% DMSO solution with or without vitamin E on the DOX lesions did not reduce ulceration. Neither antidote was toxic to mouse skin. In contrast, DMSO and the combination of DMSO plus vitamin E given intradermally in the absence of DOX proved toxic to mouse skin and produced significantly greater ulcerations than when DOX was administered alone ($P < 0.05$ by SNK multiple-range tests, table 1). Intradermal vitamin E alone was nontoxic; it also did not reduce intradermal DOX-induced ulcerations. The combination of topical DMSO plus vitamin E applied daily for 7 days to DOX-induced lesions did not reduce ulceration significantly. Also, this combination had no discernable "systemic" effect on the untreated lesions induced by 0.05 mg of intradermal DOX proximal to the DMSO-treated sites.

DISCUSSION

This study shows that topical DMSO and a DMSO-vitamin E combination do not reduce intradermal DOX-induced skin ulceration in an established mouse model (8). In addition, no "systemic" effect on nearby untreated DOX-induced lesions was observed with either regimen. These results differ markedly from positive studies with DMSO in rat (6,7) and in pig (5) skin.

In this mouse model, intradermal DMSO caused significant toxic effects in the skin, while a topically applied DMSO solution had no toxic effect. This pattern is consistent with earlier toxicologic studies of DMSO in various animal models (9). Thus, while high-dose topical DMSO applications are well-tolerated, subcutaneous and intramuscular administrations have been associated consistently with necrosis, inflammation, and edema (10–12).

Besides the purported action of DMSO to "scavenge" DOX-generated, oxygen-free radicals or to enhance penetration of tissue-entrapped DOX, it has been reported to have other diverse pharmacologic properties. For example, DMSO has been shown to provide: *(a)* locoregional analgesia (9), *(b)* anti-inflammatory effects (13) including inhibition of fibroblast proliferation and subsequent collagen synthesis (14), *(c)* potent vasodilation similar to histamine-type effects (15), and *(d)* antibacterial actions (16). This partial list helps to emphasize the difficulty in explaining the pharmacologic mechanism involved in the interaction of DMSO with any drug.

The reason for the divergence of the current results from those of earlier studies is unclear. The mouse model

used here has provided reproducible and quantifiable DOX-induced skin lesions in a series of previous studies, which have identified active local antidotal interventions. These include low doses of glucocorticosteroids (2), beta-adrenergic compounds (3), and local cold application.[4] The response of mouse skin to DMSO may differ significantly from that of rat skin or pig skin; however, mouse skin has previously shown very comparable responses to those of rabbits, rats, and human beings in standard skin irritation studies (17).

Until more testing is done and DMSO is shown to clearly reduce experimental DOX-induced skin ulcerations by a defined mechanism, our recommended approach is to apply ice and low doses of glucocorticosteroids to manage inadvertent DOX extravasations in the clinic. The intradermal or subcutaneous use of DMSO-containing solutions appears contraindicated due to their enhanced ulcerogenic effects.

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Methotrexate and Mitomycin for Patients With Metastatic Transitional Cell Carcinoma of the Urinary Tract[1]

Ian F. Tannock[2,3,*]

Sixteen patients who had received no prior chemotherapy were treated with methotrexate and mitomycin for metastatic transitional cell carcinoma of the urinary tract. Five patients achieved partial response, with a median duration of 3 months (range, 3–8), but the trial has been abandoned because of severe toxic effects in six of the 16 patients. This combination appears to be more toxic and no more effective than methotrexate alone. [Cancer Treat Rep 67:503–504, 1983]

Methotrexate is known to have activity for metastatic transitional cell carcinoma (TCC) (1,2), and we have previously reported a 39% response rate (using stringent response criteria) for 38 patients with measurable disease treated with the combination of methotrexate, doxorubicin, and cyclophosphamide (3). Unfortunately, several of our patients experienced severe mucositis and/or nausea and vomiting. Mitomycin has been reported to give a response rate of ~ 20% when used to treat metastatic TCC (4) and seems effective against superficial disease when instilled into the bladder (5). Since both methotrexate and mitomycin cause little nausea and vomiting as compared to combinations containing doxorubicin or cisplatin, we have evaluated the combination of methotrexate and mitomycin in patients with metastatic TCC.

DESIGN OF STUDY

All 16 patients had TCC of the bladder, ureter, or renal pelvis, and all had bidimensionally measurable disease as defined by physical examination, x-rays, or computerized tomographic scan (table 1). None of the patients had received prior chemotherapy. Mitomycin (15 mg/m^2, or 10 mg/m^2 in patients > 65 years of age or with extensive prior radiation therapy) was given iv every 6 weeks. Methotrexate (100 mg/m^2) was administered iv every 2 weeks, starting at the same time as mitomycin; 24 hours later, the first of four doses of folinic acid (10 mg/m^2) was given orally, and subsequent doses were administered at 6-hour intervals. Methotrexate was omitted on Days 14 and/or 28 if there was (*a*) moderate or severe myelosuppression (ie, if the polymorphonuclear leukocyte count was < 1000/mm^3 or the platelet count was < 10^5/mm^3), (*b*) mucositis, or (*c*) a > 50% increase in serum creatinine. Appropriate dosage modifications were made on second and subsequent courses if there were toxic effects.

All patients are included for evaluation of response and toxic effects. Our stringent definition of response required the following: *complete response*—the disappearance of all soft tissue lesions, normalization of any biochemical abnormalities, and recalcification of osteolytic lesions for at least 3 months; *partial response (PR)*—a > 50% decrease in the product of two diameters of *all* measurable lesions, with no progression in any nonmeasurable lesion for at least 3 months; and *no response (NR)*—all other conditions.

RESULTS AND DISCUSSION

The results of treatment are summarized in table 1. Five patients achieved PR (response rate, 31%; 95% confidence limits, 9%–54%), but only one of these patients remained in remission for longer than our minimum duration of 3 months. This patient had a slow and delayed response following two courses of treatment, which continued in the absence of further chemotherapy. His disease subsequently progressed in the pelvis, with continuing response in lung; he was then treated with radiation therapy.

Six patients had severe, life-threatening toxic effects, including a young patient in good PR who developed anemia, thrombocytopenia, pericarditis,

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TABLE 1.—Characteristics of patients treated in the study

Patient No.	Sites of disease	ECOG PS*	No. of courses	Response (duration)	Toxicity†	Reason for stopping treatment
1	Lung, bladder	1	2	PR (8 mos)	Minimal	Progression
2	Abdominal mass	1	4	PR (3 mos)	None	Progression
3	Abdominal mass, bone	2	3	PR (3 mos)	Minimal	Progression
4	Lung, nodes, bone, abdominal mass	2	2	PR (3 mos)	Severe myelosuppression and mucositis	Progression
5	Lung, bone	1	2	PR (3 mos)	Thrombotic thrombocytopenic purpura syndrome (renal failure, thrombocytopenia, pericardial effusion, pulmonary infarction)	Toxicity
6	Lung, bone	2	3	NR	None	Progression
7	Nodes, liver	2	1	NR	None	Progression
8	Nodes, skin	2	1	NR	Minimal	Progression
9	Skin	1	3	NR	Minimal	Progression
10	Lung, abdominal mass	1	3	NR	Minimal	Progression
11	Nodes, pelvic mass, bone	2	2	NR	Minimal	Progression
12	Nodes, skin	1	2	NR	Mucositis, phlebitis	Progression
13	Abdominal mass	2	1	NR	Myelosuppression, rectal bleeding	Toxicity
14	Abdominal mass, bone	2	1	NR	Severe neutropenia, renal impairment	Toxicity
15	Lung	2	1	NR	Pancytopenia, septicemia, mucositis, rectal bleeding	Toxicity
16	Liver	3	1	NR	Diffuse intravascular coagulation syndrome with thrombocytopenia, anemia, and death	Death

*Eastern Cooperative Oncology Group performance status: 0 = no symptoms, 1 = some symptoms, 2 = bedridden < 50% of the time, 3 = bedridden > 50% of the time, 4 = completely bedridden.

†Toxicity is described as minimal if there was nausea without vomiting or, for myelosuppression, if nadir granulocyte count was > 1000/mm^3 and nadir platelet count was > 10^5/mm^3.

pulmonary infarcts, and progressive renal failure requiring dialysis. She was thought to have thrombotic thrombocytopenic purpura syndrome secondary to mitomycin treatment (6,7) and did not respond to plasmapheresis. A second patient developed anemia, thrombocytopenia, and coagulation defects consistent with diffuse intravascular coagulation, but this patient had multiple hepatic metastases and the role of chemotherapy in causing this syndrome is uncertain. Four additional patients had severe mucositis and/or myelosuppression, although all of them recovered from these acute toxic effects.

My results agree with those of Body et al (8) who also reported severe and unpredictable toxic effects associated with the combination of methotrexate and mitomycin. Since this combination has high toxicity and has no demonstrated therapeutic benefit as compared to methotrexate alone or in combination with drugs that give more predictable toxicity, it should not be used further for treatment of TCC of the urinary tract.

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Phase II Study of Ftorafur in Previously Untreated and Treated Patients With Advanced Colorectal Cancer[1,2]

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Ftorafur (FT), a weak pyrimidine antagonist first synthesized in the Soviet Union, has a structure similar to 5-FUDR, and in vitro slows formate incorporation into DNA thymidine (1). During the metabolism of FT, small amounts of 5-FU are slowly released. In studies in the Soviet Union, Japan, and more recently in the United States, in which oral FT is often employed for 21 consecutive days, FT has demonstrated activity against animal and human tumors similar to that of 5-FU (2). Most American patients had received prior chemotherapy (3), often with 5-FU, with only a single previous study reported in previously untreated colorectal cancer (4). FT at doses of 2.0–3.0 g/m^2 × 5 days every 2–3 weeks produced nausea, vomiting, hypotension with rapid iv injection, and central nervous system (CNS) toxicity (ataxia, confusion, and headache), but only minimal bone marrow suppression

This phase II trial of iv FT alone in colorectal carcinoma was carried out in patients who had received either no prior chemotherapy or no prior fluorinated pyrimidines.

MATERIALS AND METHODS

Eligibility criteria included histologic confirmation of unresectable or metastatic colorectal adenocarcinoma, measurable tumor, an Eastern Cooperative Oncology Group performance score of 0–3, adequate nutrition, and informed consent.

Treatment was delayed at least 1 month after radiation therapy, chemotherapy, or major surgery. Contraindications to participation included: leukocyte counts < 4000/mm^3, platelet counts < 130,000/mm^3, previous fluorinated pyrimidine treatments, and ≥ 2500 rads to the pelvis and lumbosacral area.

FT (2.5 g/m^2/day × 5) was administered by iv infusion over 1–2 hours. Those patients with objective response (50% reduction of longest diameter product) or stable disease continued to receive treatment. Treatment was discontinued in patients with progressive disease.

RESULTS

Characteristics of the 36 patients entered in this study are summarized in table 1. Eight patients had received prior chemotherapy (cytarabine, three patients; maytansine, three; metronidazole, two; and dianhydrogalactitol and pyrazofurin, one patient each), and one patient had received prior radiotherapy.

Nausea (97%) and vomiting (94%) were nearly universal, frequently severe and prolonged, and occasionally intolerable. Vomiting recurred despite lengthening the infusion time to 2 hours and reducing the dose by 20%. Most patients had nausea and anorexia during the entire 5 days of treatment. Patients also described a wide variety of usually transient CNS symptoms (table 1) which, even when severe, subsided within a few hours. Neither significant hypotension nor drug-associated deaths were observed.

Objective responses occurred in four patients (11%), all with no prior chemotherapy, including one complete regression (3%). All patients with objective responses had symptomatic improvement. Eleven patients had disease progression in < 7 weeks, while 21 patients had periods of objective stability lasting 10–45 weeks.

DISCUSSION

While stomatitis, diarrhea, and especially hematologic toxicity are less frequently significant problems with FT treatment than with 5-FU, these advantages

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total eligible	36
Sex	
Male	20
Female	16
Median age in yrs (range): 61 (32–80)	
Performance status	
0	7
1	19
2	8
3	2
Toxicity	
Nonhematologic	
Nausea	35 (97)
Vomiting	34 (94)
Stomatitis	8 (22)
Diarrhea	4 (11)
CNS symptoms	
Dizziness (lightheadedness)	23 (64)
Headache	7 (19)
Somnolence	4 (11)
Visual changes	3 (8)
Ataxia	2 (6)
Restlessness	2 (6)
Depression	2 (6)
Hematologic (N = 31)	
Wbc count nadir < 4000 cells/mm^3	8 (26)
Platelet count nadir < 130,000 cells/mm^3	8 (26)
Response (at 10 wks)	
Complete/partial	4 (11)
Stable disease	21 (58)
Progressive disease	11 (31)

are significantly outweighed by the severe nausea and vomiting and other CNS toxic effects of iv FT. The objective responses (lasting a median duration of 5 months) observed in four of 36 (11%) patients do not suggest a therapeutic advantage for FT when compared to our previous experience with 5-FU in colorectal carcinoma (a 17% objective response rate among 359 patients) (5).

The low overall response rate, distressful and frequent nature of side effects, poor acceptance by patients, and more cumbersome method of administration than standard iv push 5-FU indicate that iv FT as a single agent is not a useful addition to the therapeutic armamentarium against advanced colorectal carcinoma.

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Phase II Trial of Aziridinylbenzoquinone (AZQ) in Patients With Refractory Lymphoma[1,2]

Peter McLaughlin,* Fernando Cabanillas, Agop Y. Bedikian, and Gerald P. Bodey[3]

Aziridinylbenzoquinone (AZQ) is a synthetic compound with good lipid and water solubility which has activity against a number of experimental tumors (1). Phase I–II trials have been promising (1–3). A minor response was seen in a patient with lymphoma at the University of Vermont (1), and partial responses were seen in two children with Hodgkin's disease (4). We report a phase II trial in patients with lymphoma.

MATERIALS AND METHODS

Adults with progressive lymphoma after previous chemotherapy and radiotherapy were eligible for AZQ. The starting dose was 30 mg/m^2 in 500 ml of normal saline over 1 hour iv every 28 days. Subsequent doses were reduced by 20% if the lowest granulocyte (polymorphonuclear leukocyte [PMN]) count was < 500/mm^3 or if the lowest platelet count was < 50,000/mm^3. Doses were increased by 20% if there was no reduction of PMN counts to < 1000/mm^3 or platelet counts to < 100,000/mm^3. Patient characteristics are listed in table 1. Standard response criteria were used (2).

TABLE 1.—Patient characteristics in 19 evaluable patients with lymphoma

Characteristic	No. of patients
Median age in yrs (range): 53 (19–77)	
Sex (male/female)	11/8
Histologic diagnoses	
Diffuse LCL (No. transformed)	9 (4)
Nodular PDL	2
Hodgkin's disease	2
Unclassified	2
Nodular LCL, lymphoblastic lymphoma, lymphocytic lymphoma, undifferentiated lymphoma	1 each
Prior therapy	
Radiotherapy	10
Chemotherapy	19
Median No. of agents: 10	
Median No. of regimens: 3	
Response to prior therapy	
CR with primary therapy	6
CR at any time	11
Never achieved CR	8

RESULTS

Twenty-one patients received a total of 45 courses of AZQ (median, two). Seven patients received three or more courses.

Two patients were inevaluable because of early death. Of the 19 evaluable patients, nine were nonresponders after one course. Of the remaining ten patients, five were nonresponders after two to three courses. Five patients [with nodular large cell lymphoma (LCL), Hodgkin's disease, diffuse LCL, diffuse undifferentiated lymphoma, and nodular poorly differentiated lymphoma (PDL)] had stable disease for 2, 3, 3, 3, and 7 months, respectively. One patient (with diffuse PDL transformed to diffuse LCL) achieved a complete response (CR) after six courses of AZQ, which lasted 6 months.

Toxicity was mainly hematologic, with thrombocytopenia being the most severe effect. Dosage escalation was done in three patients, but two of these required subsequent reductions and the other had progressive lengthening of his treatment intervals. The platelet count nadir was generally seen later (median, Day 18) than the wbc count nadir (median, Day 12). Bleeding oc-

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TABLE 2.—Hematologic toxicity with serial AZQ courses

Course No.	No. of patients (data available)	Median nadir (× 10^3 cells/mm^3)— Platelet count	Wbc count	PMN count	Treatment intervals (days)
1	19 (16)	80	2.6	1.1	28
2	10 (6)	44	3.0	1.2	28
3	7 (6)	63	3.5	1.6	44
4	5 (3)	47	2.4	0.9	28
5	3 (3)	29	2.1	1.3	40

curred in one patient, when the platelet count was 4000/mm^3. Unexplained anemia occurred in five patients, as has been noted previously with AZQ (3). There was a trend toward progressive reduction of blood cell counts with repeated courses, ie, cumulative myelosuppression (table 2). Neutropenic fever occurred in five patients; one had documented infection. Other toxic effects included nausea and vomiting in five patients and mucositis in two.

DISCUSSION

At the dose and schedule used, AZQ had little activity in this series of lymphoma patients who had failed extensive prior treatment. With only one major response (CR) among 19 evaluable patients, the likelihood is low that AZQ would have a major impact in this patient population. Moreover, myelosuppression was severe, especially thrombocytopenia.

The limited activity of AZQ in this trial is disappointing because 11 of these patients had previously achieved CR with other regimens. The prior responsiveness of lymphoma patients correlates with response to salvage regimens (5). We feel that AZQ is a drug with limited usefulness and appreciable myeloid toxicity in this patient population.

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Phase II Trial of Etoposide in Adenocarcinomas of the Upper Gastrointestinal Tract[1,2]

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Etoposide (VP-16-213) is a semisynthetic derivative of podophyllotoxin, which is postulated to have antitumor activity by mechanisms involving DNA inhibition (1). It appears to be most active in the G_2 phase of the cell cycle. In vitro studies had suggested schedule dependence with smaller, more frequent dosages being more effective than larger, more intermittent ones (2). The drug has undergone extensive clinical trials and has demonstrated antitumor activity in small cell lung cancer, leukemia, lymphoma, and testicular cancer. During its phase I trial, two of ten patients with gastric cancer had partial responses (3). In view of this encouraging activity, a phase II trial of VP-16-213 was performed in patients with advanced adenocarcinomas of the upper gastrointestinal tract.

MATERIALS AND METHODS

Thirty-eight patients with advanced adenocarcinomas of the upper gastrointestinal tract were entered into this study. All patients had measurable or evaluable disease. Measurable disease included well-defined masses in lymph nodes, subcutaneous tissue or lung, or masses measurable by computerized tomographic scan. The primary tumor at the gastroesophageal junction or in the stomach was considered to be evaluable by barium contrast studies; responses were independently quantitated by a reference radiologist (R. Heelan).

VP-16-213 was given as an iv infusion on Days 1, 3, and 5. Therapy was repeated every 3–4 weeks. The starting dosage was 120 mg/m^2/day (total, 360 mg/m^2/cycle) for good-risk patients and 100 mg/m^2/day (total, 300 mg/m^2/cycle) for poor-risk patients (heavy prior therapy or performance status of 50–70). Doses were escalated or reduced by 60 mg/m^2/cycle in the presence or absence of myelosuppression.

RESULTS

Characteristics of the patient population are shown in table 1. Three partial responses were seen among the 36 evaluable patients (8%); all occurred in previously untreated patients (three of 23; 12.5%). Two of the responding patients had gastric cancer and one had duodenal adenocarcinoma. Responses lasted 4, 5, and 5 months, respectively. Following relapse, all three responding patients were treated with the combination of 5-FU, doxorubicin, and mitomycin; one patient had a minor response and continues to receive therapy.

TOXICITY

In general, VP-16-213 was well-tolerated. Alopecia occurred frequently and nausea and vomiting occurred occasionally. The major toxic effect was myelosuppres-

TABLE 1.—VP-16-213 in gastric cancer: patient characteristics

Characteristic	No. of patients
Entered	38
Evaluable for response	36
Measurable disease	25
Evaluable disease	11
Location of primary tumor	
Esophagus or gastroesophageal junction	7
Stomach	29
Duodenum	2
Median age in yrs (range): 60 (38–72)	
Median performance status (range): 70 (30–90)	
Males/females	26/12
Prior therapy	
None	24
Chemotherapy	14

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sion. Six patients had severe leukopenia, with wbc count nadirs < 1500/mm^3 on at least one occasion. Thrombocytopenia was less severe (only three patients had platelet counts < 50,000/mm^3). Two patients developed fever and presumed sepsis during the nadir period which responded to antibiotic therapy.

DISCUSSION

Although declining in incidence, gastric cancer remains a major therapeutic problem in the United States. In the presence of metastatic disease, median survival among patients who do not respond to chemotherapy is usually 3–4 months in duration. Although newer combinations of conventional agents (such as 5-FU, doxorubicin, and mitomycin) have been reported to induce remissions in 35%–45% of patients, this still means that most patients with gastric cancer do not respond to the most effective drug combinations. Clearly, newer active systemic agents are needed. VP-16-213 was chosen for phase II testing because of the encouraging activity seen in gastric cancer during its phase I trial. Modest antitumor activity in previously untreated patients was noted in the current study. The mode of action of VP-16-213 is different from that of other agents commonly used in the treatment of gastric cancer, suggesting a role for the drug in combination therapy. Recent studies in gastric cancer have also noted modest activity for cisplatin (4); a combination using VP-16-213 and cisplatin may be an attractive one for future studies in adenocarcinoma of the upper gastrointestinal tract.

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PCNU in Advanced Adenocarcinoma of the Ovary: A Phase II Study[1,2]

David A Decker,* Michael J. Samson, and Laurence H. Baker[3,4]

PCNU, an *N*-(2-chloroethyl)-*N*-nitrosourea, was selected for clinical trials because of activity against a variety of animal tumors (1). In phase I trials the dose-limiting toxic effect for a variety of schedules was myelosuppression (2–4). Three other nitrosoureas, carmustine, lomustine, and semustine, have received limited trials in advanced ovarian cancer (5). Response rates for these agents are reported to be 6%, 19%, and 0%, respectively (5). We have conducted a phase II investigation of PCNU in patients with stage III and IV (International Federation of Gynecology and Obstetrics) adenocarcinoma of the ovary who had previously been treated with chemotherapy.

Between November 4, 1980, and December 7, 1982, 15 patients were entered in this study (table 1). One patient was not evaluable for response because of early

TABLE 1.—Patient characteristics, response, and toxicity

	Chemotherapy dose (mg/m²)	
	75	100
No. of patients		
Entered	7	8
Evaluable for toxicity	7	8
Evaluable for response	6	8
With prior chemotherapy	7	8
Age in yrs		
Range	37–69	53–83
Median	60	62
Karnofsky performance score		
Range	40–90	60–90
Median	80	70
Best response (No. of patients)		
Stable disease (duration in wks)	1 (14)	4 (7,12,18,20)
Disease progression	5	4
Survival in wks		
Range	3–24	6–32
Median	7	8
Myelosuppression		
Wbc count nadir ($\times 10^3$ cells/mm³)		
Range	3.0–9.7	1.6–7.2
Median	5.9	3.5
Platelet count nadir ($\times 10^3$ cells/mm³)		
Range	28–203	13–381
Median	64	139

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death after 3 weeks from other medical problems. All patients had histologically confirmed adenocarcinoma of the ovary. Patients had not received hormonal or cytotoxic therapy for > 4 weeks prior to entering the study. Pre-evaluation studies included history and physical examination, performance status, tumor measurement, chest radiogram, cbc, platelet count, creatinine, BUN, liver function studies, computerized axial tomographic scan/ultrasound (if indicated). These studies were repeated every 3 weeks with weekly cbc and platelet count.

Responses were defined as follows: *complete response*—the complete disappearance of all measurable and clinically recognizable tumor for a minimum of 4 weeks; *partial response*—a ≥ 50% decrease in the products of the two longest perpendicular diameters of all measurable disease for a minimum of 4 weeks; *stable disease*—no change of measurements or a change less than partial remission or disease progression; and *disease progression*—a ≥ 25% increase in the product of two diameters of measurable disease or the appearance of new lesions. Measurable disease was a pelvic or abdominal mass in 13 patients and supraclavicular nodes in two patients.

Good-risk patients (wbc count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, radiation to < 50% of the functioning bone marrow, and ≤ 2 times normal serum bilirubin, alkaline phosphatase, SGOT, LDH, or creatinine) received 100 mg/m^2 of PCNU every 6 weeks. Poor-risk patients (wbc count < 4000 but ≥ 3000/mm^3, platelet count < 100,000 but ≥ 75,000/mm^3, radiation to > 50% of the functioning bone marrow, and > 2 times normal serum bilirubin, alkaline phosphatase, SGOT, LDH, or creatinine) received 75 mg/m^2 of PCNU every 6 weeks.

Patient characteristics are shown in table 1. There were no complete or partial responses. Stable disease was observed in five patients (table 1). Only six patients received more than one course of PCNU. One patient received two courses despite disease progression after the first course of PCNU, one patient received two courses of PCNU with stable disease, and four patients received three courses of PCNU with stable disease. The remaining patients had disease progression either within or at 6 weeks from the first day of PCNU. All patients have died except for three who are alive with progressive cancer (table 1).

Gastrointestinal and hematologic toxicity was observed. Seven patients had mild to moderate vomiting and two had severe vomiting at the higher (100 mg/m^2) dose level. Mild to moderate diarrhea occurred in three patients. Myelosuppression was reversible and mild in all patients but one, with a wbc count nadir of 1600/mm^3 and a platelet count nadir of 13,000/mm^3 requiring platelet transfusions. Cumulative myelosuppression was suggested in four of the six patients receiving more than one course of PCNU. During Course 2 we observed a platelet count nadir of 64,000/mm^3; during Course 3 we observed platelet count nadirs of 67,000 and 68,000/mm^3 and wbc count nadirs of 1600 and 3900/mm^3. Other organ site toxic effects were not observed.

We conclude that PCNU, in the dose and schedule chosen, induces a true response rate of < 20% in previously treated patients with advanced adenocarcinoma of the ovary, with a false-positive or false-negative error of < 0.05 (6).

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Cisplatin for Adrenal Cortical Carcinoma[1]

Carcinoma of the adrenal cortex is an extremely rare neoplasm, with an annual incidence in the United States population of 0.2/100,000 (1). While surgical resection can control local disease, tumor extension or metastases frequently are found at diagnosis. Adrenal cortical carcinoma is relatively radio-resistant, and the evaluation of antineoplastic agents for therapy has been limited. Mitotane, the most thoroughly tested and effective agent against inoperable disease, induces objective tumor regression (including non-hormone-producing tumors) averaging 10 mos in 34%–61% of the patients and biochemical improvement of elevated steroids in 50%–85% (1). There have been few reports of efficacy of chemotherapeutic agents, and no single agent or combination-drug regimen has been found to be of consistent benefit against this highly refractory tumor. Some antitumor activity has been noted in single patients given carmustine, porfiromycin, doxorubicin, peptichemio, doxorubicin and bleomycin, 5-FU with mitotane or methotrexate, and the COMB (cyclophosphamide, vincristine, semustine, and bleomycin) regimen (2). Recently, Tattersall et al (3) have reported four responses among four patients given cisplatin (DDP). Five patients at the Memorial Sloan-Kettering Cancer Center (MSKCC) with bidimensionally measurable, metastatic adrenal cortical carcinoma were treated with DDP, forming the basis of this report.

All patients had a complete history, physical examination, pathologic confirmation by the Department of Pathology, MSKCC, and evidence of bidimensional lesions at the time of initial therapy. Biopsy of a metastatic lesion was obtained in four of five patients < 1 mo prior to DDP therapy. Cbc, platelet count, creatinine clearance, biochemical screening profile, serum and urinary cortisol, 17-hydroxy and 17-ketosteroid levels, chest x-rays, liver and bone scans, iv pyelograms, and computerized tomographic scans of the abdomen and/or chest were obtained. Serum electrolytes and BUN, magnesium, and creatinine levels were obtained daily for 3–5 days after each dose. Cbc was repeated on Days 7, 10, and 14. X-rays and computerized tomographic scans were reviewed independently (R. C. Watson). Cytotoxic agents, including mitotane, were discontinued at least 4 wks prior to DDP. There were three males and two females, with a median age of 28 yrs (range, 24–50). The median Karnofsky performance score was 70% (range, 50%–80%). Three patients had simple and two had radical adrenalectomies. The median time in mos from diagnosis to metastasis was 0.5 (range, 0–21) and from metastasis to DDP was 6 (range, 3–7). All patients had received mitotane, two had received prior chemotherapy, and all had a hormone-producing tumor. Three patients had lung and abdominal masses, one had subcutaneous lesions, and one had liver metastasis. DDP at a dose of 120 mg/m^2 with mannitol diuresis was administered in 25–30 mins every 4 wks in four patients iv, and in one (who had only hepatic metastases) intra-arterially via a catheter placed in the hepatic artery by a percutaneous transfemoral approach. After two courses, DDP was given in a split-dose schedule (60 mg/m^2/day $\times$ 2) via the side arm of the Infusaid pump which had been connected to a catheter placed surgically in the hepatic artery. An adequate trial was defined as two doses. Partial remission was defined as a $> 50\%$ decrease in the summed products of the largest perpendicular diameters of all lesions, and a $> 50\%$ decrease in all abnormally elevated biochemical parameters for > 1 mo; minor response was defined as a 25%–50% decrease in all parameters; disease stabilization was defined as a $< 25\%$ increase or a $< 25\%$ decrease for > 3 mos; and disease progression was defined as a $> 25\%$ increase in measurable disease or a mixed response. Duration of response was measured from initiation of protocol. Karnofsky performance scale was used to evaluate subjective changes. Myelosuppression was defined as a wbc count < 3000/mm^3 and/or a platelet count $< 100{,}000$/mm^3. Nephrotoxicity indicated an increase in the serum creatinine level to $> 100\%$ of baseline values within 3 days after DDP, or a $> 25\%$ decrease in the creatinine clearance.

RESULTS AND DISCUSSION

Four of five patients had an adequate trial; one died from progressive disease < 2 wks after DDP. Although there were no complete or partial responses, one patient had a minor response for 7 mos and another had disease stabilization for 4 mos. All patients have died. Nausea and vomiting was universal. One patient, who had had prior chemotherapy, experienced prolonged

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myelosuppression for 5 wks, and another had a severe neuropathy after six doses.

Tattersall et al (3) observed four responses in four patients with metastatic adrenal cortical carcinoma, two of whom had hormone-producing tumor. One patient had received prior irradiation. Three patients had received mitotane previously (one concomitantly with DDP); one of the three had responded, but none had had prior cytotoxic drugs. Doses of DDP were 20 mg/m^2/day × 5 (one patient), 100 mg/m^2 on Day 1 (two), and 30 mg/m^2/day × 4. Regression of liver, lung, or intra-abdominal metastases was noted within 3 wks and persisted for 4–9 mos. Median survival was 12 mos (range, 7–14) from diagnosis to metastasis. Of note, two patients with a non-hormone-producing tumor responded. Mild ototoxic effects were observed in two patients and renal dysfunction was observed in two. Merrin (4) reported a 50% reduction in the size of a single pulmonary metastasis lasting 6 mos in one patient with adrenal cortical carcinoma given DDP at a dose of 1 mg/kg/wk × 6 and then every 3 wks thereafter. In a drug-oriented phase II trial of DDP (20 mg/m^2/day × 5 every 4 wks), one patient with an adrenal cancer had an intra-abdominal mass decrease by 25% for 3+ mos (5). Haq et al (2) and Spiers (6) reported disease stabilization for 2 and 7 mos in two of three patients, respectively.

While DDP may be an active agent, further trials are needed to define its activity more precisely. No single cancer center or cooperating oncology group has sufficient numbers of cases to determine the true efficacy of a drug in this resistant tumor. Only through a central resource such as the National Cancer Institute, which could initiate and support on a case-entry basis an intergroup cooperative phase II trial, can progress be made.

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Comparison of 5-FU and Doxorubicin in the Treatment of Carcinoma of the Bladder

This study is a randomized, prospective phase II clinical trial of patients with far-advanced carcinoma of the bladder. The purpose of the study was to help define the roles of doxorubicin and 5-FU in the treatment of bladder cancer.

PATIENTS AND METHODS

One hundred and eight patients were entered in the study; 91 were evaluable for toxicity and drug effect. Fifteen percent of the patients entered were not evaluable because of inadequate renal function, inadequate measurable disease, or death before treatment was initiated. Each evaluable patient was randomized to receive either doxorubicin at a dose of 60 mg/m^2 iv every 3 weeks (40 mg/m^2 if previously irradiated) or 5-FU at a dose of 600 mg/m^2 iv weekly (400 mg/m^2 if previously irradiated). The maximum dose of doxorubicin was not to exceed 550 mg/m^2. A minimum of 3 weeks in the study was considered necessary for an adequate trial, and all patients with disease progression at any time after 3 weeks were crossed over to the alternate therapy. Patient characteristics included an Eastern Cooperative Oncology Group (ECOG) performance status

of 0–3. The median age was 66 years. There was a predominance of males (79%) as compared to females (21%). Thirty-five percent of all patients had not received any prior radiation therapy; however, 48% had received radiation therapy to the primary site only, 8% had received radiation therapy to metastatic sites, and 9% had received radiation therapy to both the primary and metastatic sites. No patient had received prior chemotherapy.

TOXICITY

Drug-related toxicity was coded using ECOG standards. Adequate drug doses were delivered with acceptable toxicity, which was generally mild to moderate with vomiting and myelosuppression being the most common and occurring equally between the two treatment groups. A significant number of patients (62%) experienced alopecia during treatment with doxorubicin. One patient developed cardiac toxic effects during treatment with doxorubicin, which resulted in fatal cardiomyopathy.

RESULTS

Standard ECOG criteria for response were employed. Table 1 indicates the response rates for both the initial regimen and the crossover regimen. The overall response rate for doxorubicin was 9%, with an estimated median survival of 20 weeks. The overall response rate for 5-FU was 14%, with an estimated median survival of 31 weeks. Neither response rate nor survival time showed any statistically significant difference between the treatment groups. Survival was significantly better for ambulatory patients (ECOG 0–1) as compared to nonambulatory patients (ECOG 2–3). Women survived significantly longer than men.

DISCUSSION

This study of 91 evaluable patients suggests that doxorubicin and 5-FU are not very effective agents in the treatment of carcinoma of the bladder. Most authors who have reviewed the available literature also conclude that the reported chemotherapy for bladder malignancy has not been effective in terms of response rate, response duration, or survival (1–3). No single agent or combination of agents can be considered standard therapy for this malignancy at this time (1). Combinations of drugs, however, particularly those including cisplatin, are beginning to show more promise in several early trials (1,3,4). In particular, the response rates of some of these combinations (doxorubicin + cisplatin + cyclophosphamide) are ranging from 44% to 90%, with improved response durations and survival (1).

TABLE 1.—Response to 5-FU and doxorubicin in treatment of bladder cancer

Response	No. of responses	
	Doxorubicin	5-FU
	Initial regimen	
Complete	0	2 (4%)
Partial	5 (11%)	5 (11%)
No change	11 (24%)	6 (13%)
Disease progression	29 (64%)	33 (72%)
Total	45	46
	Crossover regimen	
Complete	1 (5%)	0
Partial	0	2 (11%)
No change	5 (24%)	3 (17%)
Disease progression	15 (71%)	13 (72%)
Total	21	18

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[1]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Wuesthoff Memorial Hospital, Rockledge, FL 32955 (*E. W. Knight*); Dana-Farber Cancer Institute, Boston, MA (*M. Pagand*, CA-23318); Mayo Clinic, Rochester, MN (*R. G. Hahn*, CA-13650); and Albany Medical College, NY (*J. Horton*, CA-06594).

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Orthostatic Hypotension as a Complication of Hexamethylmelamine Antidepressant Interaction

Hexamethylmelamine (HMM) is currently widely used in experimental chemotherapy because of its broad phase II activity against common tumors of the ovary, lung, and bladder, with possible activity against additional tumors. Antidepressant medications have been used with increasing frequency in cancer patients, both in the treatment of intercurrent depressive states and in the management of pain. We report four instances of symptomatic, potentially life-threatening orthostatic hypotension in patients treated with both HMM and antidepressants of the tricyclic or monoamine oxidase inhibitor classes.

The patients ranged in age from 62 to 67 years, with primary tumors of the ovary, lung, colon, and one unknown primary, metastatic to the liver. All patients received combination chemotherapy, with no agent other than HMM common to all patients. Two patients had a long history of successful treatment with phenelzine (60 mg; Patient 1) or imipramine (150 mg; Patient 2) prior to their first treatment with HMM (200 and 250 mg/m^2 for 14 days, respectively). Both patients had incapacitating dizziness during the first week of HMM treatment and one patient had a syncopal episode upon standing. Blood pressure measurements were (sitting:standing): 80/60:50/30 (Patient 1); and 110/78:86/64 (Patient 2). The two other patients had each received several courses of HMM (Patient 3, 150 mg/m^2 and Patient 4, 250 mg/m^2) and subsequently started to receive 50 mg of amitriptyline or imipramine. Both patients had severe lightheadedness within several days of taking the antidepressant and HMM in combination. Blood pressure for Patient 3 was 110/64:60/40, while Patient 4 stopped his imipramine 1 week prior to clinical reevaluation. All four patients became asymptomatic within 24–96 hours after discontinuing the antidepressant. Patient 2 continued to manifest prominent depressive symptoms and began to receive nortriptyline at a dose of 50 mg daily. Blood pressure was 136/90:124/80 with nortriptyline alone and 140/80:100/80 7 days after adding HMM at a dose of 200 mg/m^2. No symptoms were reported.

While neurologic side effects of HMM have been regularly noted (1,2), we are not aware of any reports of orthostatic hypotension due to HMM, nor have clinical symptoms suggesting orthostasis (dizziness, falls, and ataxia) been apparent in our experience with this agent. A sample of 40 consecutive patients taking HMM without antidepressants reported no symptoms suggestive of orthostatic hypotension. Orthostatic hypotension is a common non-dose-dependent side effect of antidepressant medications, affecting approximately 25% of psychiatric patients treated with these agents, with about 10% of the patients experiencing clinically significant symptoms (3,4). In addition to the four patients described, two other patients known to be taking HMM in combination with antidepressants were identified. On retrospective questioning, one patient (age, 65 years) reported dizziness while the other (age, 40 years) reported nonspecific symptoms of discomfort with the combined regimen.

Our observations suggest that the combination of HMM and antidepressants may be associated with a high incidence of clinically significant and potentially life-threatening orthostasis. It remains to be determined if this risk is restricted to older patients, an association which has been found in some but not all studies with antidepressants alone (3). The chemotherapist should be aware of this possible drug interaction. It is potentially life-threatening and avoidable. Additional reporting is required to define the incidence of this apparent drug interaction.

There is evidence that nortriptyline may be less likely to produce orthostatic hypotension than other antidepressants (3,5). It may therefore be the drug of choice if an antidepressant is required in patients receiving HMM. It is of note that Patient 2 tolerated a nortriptyline-HMM combination without clinical symptoms, although a postural drop in systolic blood pressure was found.

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Gynecologic Laser Surgery

Maui, Hawaii, July 16–23, 1983

This continuing education program offering meets the criteria for AMA category 1 credit for physicians and the California and Nevada Boards of Registered Nursing for nurses.

Tuition is $285 for physicians and $230 for nurses.

For more information, contact: Symposia Medicus, 2880 Shadelands Dr, Suite 404, Walnut Creek, CA 94598 (telephone 415-935-7889).

Predictive Drug Testing on Human Tumor Cells

Zurich, Switzerland, July 20–22, 1983

An international conference on predictive drug testing on human tumor cells will be held at the University Hospital of Zurich. The conference will deal with newly developed techniques for individualizing treatment of cancer patients. The program will include lectures by invited speakers, competitive papers, and a poster session. The deadline for receipt of abstracts is April 15, 1983.

For further information, contact: V. Hofmann, MD, Division of Oncology, University Hospital, 8091 Zurich, Switzerland

Progress and Controversies in Oncological Urology

Noordwijkerhout, The Netherlands, September 8–10, 1983

Organized by the Departments of Urology and Medical Oncology of the Erasmus University, Rotterdam, and the St. Radboud University Hospital Nijmegen, this symposium is designed for urologists, medical oncologists, radiotherapists, and others interested in an up-to-date discussion of oncological urology.

The meeting is designed to provide participants with the results of the most recent advances in the diagnosis and treatment of urological malignancies. The program will consist of two parts, formal presentations with subsequent round tables and seminars. The seminars are designed to give participants a chance to raise questions and have the opportunity to discuss pertinent problems with the experts after short introductions.

The organizing committee includes: Frans M. J. Debruyne, Karl H. Kurth, Fritz H. Schröder, Ted A. W. Slinter, D. J. Theo Wagener, and Alphons F. G. V. M. Ypma. Registration will be limited to 300 participants.

For further information, contact: Mrs. E. Vélu-Mathôt, Secretary, Department of Urology, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands (telephone: (010)-633095 or (010)-633607).

Cancer Chemotherapy and Selective Drug Development

Brighton, England, October 24–28, 1983

This Tenth International Symposium on the biological characterization of human tumors is a multidisciplinary forum for the discussion of contemporary problems in cancer chemotherapy.

The plenary sessions include clinical topics: improved drug combinations, high-dose therapy, bone marrow transplantation, new drugs, drug resistance, treatment of specific cancer, immunotherapy, endocrine therapy, and control of infection; and preclinical topics: experimental models, drug resistance, pharmacokinetics, targeted chemotherapy, biological response modifiers, new drug development, and radio/chemosensitisation.

The study/workshop groups cover topics in medicine, Control fo Pain and Emesis in Cancer Patients; nursing, Bridging the Gap Between Science and Care; biochemistry, Chromatin as a Target in Cancer Chemotherapy; and hormone receptors, Hormone Receptors and Their Role in Directing Therapy.

Proffered papers and posters are invited on the themes of the symposium. Registration is restricted to 350 active participants.

For further information, contact: CCHTI Secretariat, Institute of Cancer Research, Block E, Clifton Ave, Belmont, Sutton, Surrey SM2 5PX, England.

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgery and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 660 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

New Perspectives in Human Lymphoma

Houston, Texas, November 9–12, 1983

This 27th annual clinical conference, held at the Shamrock Hilton Hotel, will focus on various controversial aspects of the diagnosis and management of lymphomas. Topics to be covered are pathogenesis and etiology, staging, and the use of various modalities of therapy, including, radiation, chemotherapy, immunotherapy, and bone marrow transplantation.

Speakers include: C. Berard, D. Bergsagel, C. Bloomfield, G. Bonadonna, R. Fisher, E. Frei, N. Gutensohn, M. Kadin, A. Levine, I. Magrath, L. Nadler, and L. Prosnitz. Conference co-chairpersons are Drs. Richard Ford, Lillian Fuller, and Fredrick B. Hagemeister.

For more information, contact: Office of Conference Services, Box 18, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030 (telephone: 713-792-2222).

International Conference on Head and Neck Cancer

Baltimore, Maryland, July 22–27, 1984

The American Society for Head and Neck Surgery and The Society of Head and Neck Surgeons are sponsoring this conference to be held at the Hyatt Regency Hotel in Baltimore's Inner Harbor. Topics will include basic research in virology, biochemistry, immunology, cell and tissue culture and other areas; etiologic factors; carcinogens; viral studies; epidemiology; early detection; diagnosis and staging; results of surgery, radiation therapy and chemotherapy; modifications of standard treatments; new treatment modalities; nutrition; rehabilitation; reconstruction; design of clinical trials; and statistical considerations.

The conference will provide dissemination of information through didactic sessions, symposia, panels, workshops, cine discussions, and scientific exhibits. Abstracts are invited for oral presentations and scientific exhibits.

For further information, contact: The Program of Continuing Education, University of Maryland School of Medicine, 10 S Pine St, Baltimore, MD 21201 (telephone 301-528-3956).

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